PHARMACOLOGIC-FUNCTIONING WATER AND USAGE OF THE SAME

TECHNICAL FIELD

The present invention relates to a new pharmacologic-functioning water
5 demonstrating pharmacologic function without any side effects and including
antioxidant-functioning water used as an active principle containing
hydrogen-dissolved water, which is made up of molecular hydrogen used as a substrate
that is included in raw water, and a precious metal colloid, which is included in the
hydrogen-dissolved water and catalyzes the breaking reaction of the molecular
10 hydrogen into a product of atomic hydrogen, and usage of the same.

BACKGROUND ART

For living organisms, oxygen is a double-edged sword. It has been pointed out
that while oxygen is used to procure energy by oxidizing nutrients and/or performing
various oxygen-added reactions essential for living organisms, there is a risk that leads
15 to various types of constitutional disturbances emanating from such oxidizing power.

In particular, a metabolism-produced active oxygen radical and a free radical
such as nitric oxide (NO) are highly reactive atoms and molecules, which have unstable
unpaired electrons, and try to maintain their stability by capturing or transferring one
more electron. Generally, four types: superoxide anion radical ($\cdot O_2^-$), hydroxy radical
20 ($\cdot OH$), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$) are called active oxygen
radicals. Hydroperoxy radical ($HO_2\cdot$), peroxy radical ($LO_2\cdot$), and alkoxy radical ($LO\cdot$)
are thought as broad active oxygen radicals. In the following description, these active
oxygen radicals may be generically referred to as 'active oxygen species'. In addition,
what are referred here as active oxygen species and free radicals such as nitric oxide
25 (NO) may simply be generically called 'radicals'.

Such active oxygen species are used for bacteria-killing action by cells and the
like in living organisms, intracellular signal transduction mechanism through redox
control, degradation of unnecessary proteins, apoptosis, and the like, or are regarded as
contributing thereto. For example, in the case of an inflammatory reaction,
30 macrophages, which are immune cells, generate by themselves active oxygen, which is a
means of cell-damaging action for attacking bacterial cells and the like of foreign bodies.

However, excessive amounts of active oxygen species generated by oxidative
stress are extremely harmful to the living organisms due to high reactivity. Another
active oxygen species is produced in a circumstance including the iron ion or the copper
35 ion as a by-product from the active oxygen species (radical chain reaction). It is

becoming clear that the active oxygen species produced in such accelerating manner involves various health problems and diseases through damaging cells and DNA, and producing lipid peroxide, a factor that accelerates the aging process. In particular, the hydroxy radical ($\cdot$OH) is the highest reactive active oxygen specie, which has large

5 injurious effects such as cell damage. Furthermore, it has been confirmed that a hydroxy radical ($\cdot$OH) is produced and is involved in inflammation of skin due to ultraviolet rays (UV). It is known that viral infections have toxic effects on living organisms since, as a result of excessive immune responses of infected persons, more active oxygen species than the amount necessary for keeping balance in the organisms

10 are produced.

Active oxygen species that have such toxic effects on living organisms are normally scavenged in living organisms with an enzyme such as superoxide dismutase (SOD) or catalase.

However, it has been found that if balance in the organism is upset, for

15 example by factors such as stress, alcohol, smoking, strenuous exercise, or aging, SOD levels decrease and lipid peroxide increases because of the active oxygen species. This brings on various health problems such as cerebral infarctions, heart attacks, arteriosclerosis, diabetes, cancer, strokes, cataracts, stiff shoulders, over sensitivity to cold, high blood pressure, and senile dementia, as well as problems of degradation in

20 physiological functions of the organism, or of degeneration in cosmetic appearance, such as age spots, freckles, and wrinkles.

Free radical scavenging agents and anti-oxidizing agents such as ascorbic acid, alpha-tocopherol, cysteine, glutathione, ubiquinone, butyl hydroxyanisole (BHA), and butyl hydroxytoluene (BHT) are known as substances for overcoming such active

25 oxygen species-derived problems.

(PROBLEMS TO BE SOLVED BY THE INVENTION)

Nevertheless, since such anti-oxidizing agents are chemically synthesized compounds, there are problems including remaining doubts as to the safety of such substances on the antioxidation subject (i.e., living cells) when used in large quantities.

30 Another problem is the fact that these and similar anti-oxidizing agents become oxidized themselves through the process of reducing other substances and raises questions as to the safety (for example, radical chain reaction) of such by-product oxides on the antioxidation subject.

Therefore, development of innovated technology demonstrating pharmacologic

function without any side effects clearly different from ready-made cures inevitably accompanied by side effects is eagerly anticipated.

The present invention has been made in order to solve such problems and aims to provide pharmacologic-functioning water demonstrating pharmacologic function without any side effects and including antioxidant-functioning water as an active principle containing hydrogen-dissolved water, which is made up of molecular hydrogen used as a substrate that is contained in raw water, and a precious metal colloid, which is included in the hydrogen-dissolved water and catalyzes the breaking reaction of the molecular hydrogen into a product of atomic hydrogen, and usage of the same.

DISCLOSURE OF THE INVENTION

Before giving a general description of the invention, the history of how the inventors have arrived at the present invention is described.

(1) History of Inventive Idea

In the previously filed and republished patent application No. WO99/10286, the contents of which are incorporated herein by reference, the applicants of the present application disclose an electrolytic cell and an electrolyzed water generation apparatus capable of independently controlling the hydrogen ion exponent (hereafter referred to as 'pH') and the oxidation/reduction potential (hereafter referred to as 'ORP'). A synopsis of the aforementioned application is given forthwith. Namely, a reducing potential water generation apparatus has: an electrolytic chamber into which raw water to be electrolyzed is supplied; at least one membrane which separates inside the electrolytic chamber from outside thereof; at least a pair of electrode plates provided inside and outside the electrolytic chamber, respectively, and sandwiches the membrane; and a power supply that supplies a voltage between both electrodes, wherein the electrode plate provided inside the electrolytic chamber is given as the cathode and the electrode plate provided outside the electrolytic chamber is given as the anode; wherein the electrode plates provided outside the electrolytic chamber are provided in contact with the membrane or leaving a slight space. On the cathode side in the apparatus, without significantly changing the pH of the raw water, electrolyzed reducing potential water (hereafter, also referred to as 'reducing potential water') is generated having an ORP that is significantly lowered to a negative value. In the following, unless not specifically stated otherwise, 'electrolysis processing' means carrying out continuous-flow electrolysis processing using the above-mentioned reducing potential water generation apparatus under electrolysis conditions of a 5 A constant current and

flow rate of 1 L/min.

The inventors herein arrived at the present invention during performance evaluation testing of reducing potential water generated with the reducing potential water generation apparatus described above.

5   Here, the reducing potential water has a negative ORP value, and also shows an ORP value corresponding to the pH that exceeds a predetermined value. Whether or not the ORP value exceeds the predetermined value may be determined through the following Nernst equation (approximate equation):

$$ORP = -59pH - 80(mV) \quad ...(Nernst \ equation)$$

10   As shown in FIG. 1, this equation shows there is a proportional relationship between the pH and ORP (the ORP value falls towards negative as the pH falls towards the alkaline side). Here, the fact that the ORP value corresponding to pH shows a value that exceeds the predetermined value means that the ORP value is lower than the value according to the Nernst equation described above. It is given here that water meeting such conditions is called reducing potential water. For example, substituting pH 7 into the Nernst equation above gives an ORP of approximately –493 (mV). In other words, at pH 7, water having an ORP of approximately –493 (mV) or lower corresponds to reducing potential water. However, some difference definitely exists in the dissolved hydrogen concentration within the category of reducing potential water defined here, but this is described later together with the quantitative analysis method for this dissolved hydrogen concentration.

Therefore, a considerable amount of high-energy electrons is included in the reducing potential water. This is clearly seen when measured with an ORP meter. The ORP is an indicator showing the proportions with which oxidizing material and reducing material exist in the test water, and generally uses units of millivolts (mV). Generally with an ORP meter, a negative ORP value is observed when the measurement electrode takes a negative charge, and conversely, a positive ORP value is observed when the measurement electrode takes a positive charge. Here, in order for the measurement electrode to take a negative charge, high-energy electrons must be included in the test water. Accordingly, the fact that ORP value shows a negative value having a large absolute value can be said as meaning that the test water includes high-energy electrons.

At this point, illumination testing using a light emitting diode (hereafter abbreviated as 'LED') was carried out for performance evaluation showing to what

extent high-energy electrons are included in the reducing potential water. This used the principle behind batteries. More specifically, reducing potential water having an exemplary ORP of approximately −600 (mV) and tap water having an exemplary ORP of approximately +400 (mV) were poured into the cathode chambers 205 and anode

5   chambers 207, respectively, in a testing cell 209 configured with alternating platinum or similar electrodes 201 and membranes 203, and having about three cathode chambers and three anode chambers. Continuous illumination of an LED 211 was observed when the minus end of the LED 211 was connected to the electrode in contact with a cathode chamber 205 and the plus end of the LED 211 was connected to an anode

10   chamber 207. This means that current is flowing from the anode of the cell 209 towards the cathode, and moreover, the fact that current is flowing means that electrons are flowing. At this point, taking into consideration the fact that the electrons flowing through the LED 211 are flowing from the cathode of the cell 209 to the anode, the included high-energy electron groups in the reducing potential water are quantitatively

15   evaluated through testing.

As reference examples, alkaline electrolyzed water generated by a commercially available electrolyzed water generation apparatus (for example, ORP of approximately −50 mV), or natural mineral water, etc, was poured into the cathode chambers and tap water was poured into the anode chambers. However, in this case,

20   continuous illumination of the LED was not observed when the minus end of the LED was connected to the electrode in the cathode chamber and the plus end of the LED is connected to the anode chamber in a manner similar to that described above. This is thought as happening because not enough high-energy electron groups to illuminate the LED are included in the existing alkaline electrolyzed water or natural mineral water.

25   In addition, even if flow were to be reduced and the ORP value shifted significantly towards the negative with a commercially available electrolyzed water generation apparatus, should the absolute value of the ORP value occurring at the pH level at that time be small in accordance with the above-mentioned Nernst equation, no illumination of the LED would naturally be observed. With, for example, the

30   commercially available electrolysis generation device, even if the pH is approximately 10 and the ORP value is in the range of −500 to −600 (mV) as a result of reducing the flow, since the ORP value as a percentage of the pH level becomes small, it may be weak in terms of the electron energy, and as long as ORP value fails to be brought down to at least approximately -670 (mV) or lower when the pH level is approximately 10, it is

impossible to illuminate the LED.

In addition, there are several varieties of LEDs. In particular, when a diode showing, for example, a blue or green color that requires a high inter-terminal voltage of approximately 3V or higher was used, continuous illumination of such diode was observed when using a cell 209 having each chamber arranged in a three-layer alternating structure as described above.

Therefore, as eager research progressed on the industrial applicability of having high-energy electrons included in reducing potential water, a hint was received that wondered if it was possible that the reducing potential water had 'latent reducing power'. In particular, the reducing potential water had quite strong reducing power since the ORP value had fallen to a appreciably negative value that was significant enough cause the LED to illuminate, which led to the feeling that if this reducing power be could tapped there may be applications over a wide range of industrial fields including health care, manufacturing, food, agriculture, automobile, and energy.

What state this 'latent reducing power' is in is now described.

For instance, if a reducing agent such as vitamin C (ascorbic acid) is added to ordinary tap water, and thereafter an oxidizing agent is further added, the reducing agent immediately reduces the oxidizing agent. On the other hand, if an oxidizing agent is added to reducing potential water, the oxidizing agent is not immediately reduced at all. Conditions at this point may include both the significant negative ORP value for the reducing potential water remaining the same, as well as the oxidizing agent also maintaining the same conditions. At this point in time reducing power has not yet been exhibited.

That is, no matter how much the high-energy electrons try to exist in the reducing potential water, or to put it another way, no matter how large and negative the value of the ORP is, it comes up against the fact that the reaction where electrons are immediately released from the reducing potential water to reduce the oxidizing agent does not occur. Therefore, it was thought that the magnitude of the electron energy included in the reducing potential water and how easily the electrons are released or the exhibition of reducing power are probably two separate issues.

So what should be done to make the reducing potential water exhibit reducing power? As the inventors continued with their eager research into this proposition, they were inspired by the idea of using some sort of catalyst. While there are many types of catalysts, with the particular premise of for instance use in living organisms,

the idea was conceived that some sort of enzyme or a precious metal colloid (minute particle of a precious metal cluster), which is described later, might be used as the catalyst.

Here, the particular mention of an enzyme is for an enzyme-acting substance that is a chemical reaction catalyst, and the activity of the enzyme is measured by the speed of the catalyzing reaction. In the case of catalyzing the reaction of A☐ B, A is the substrate and B is the product. Applying this to the case of the present invention, the molecular hydrogen included in the hydrogen-dissolved water corresponds to the substrate, and the active hydrogen corresponds to the product. Also, it is thought that the working-action mechanism of such enzyme can be described in the following manner.

It is assumed here that it is necessary for the high-energy electron group included in the reducing potential water to come into contact with the oxidizing agent and reduce this oxidizing agent. There is an energy wall that this electron group included in the reducing potential water must surpass in order for the electron group to migrate to the oxidizing agent. This energy wall is commonly called a 'potential barrier', 'activation energy', or the like. The higher this energy is, the higher the height of the wall that needs to be surpassed becomes. Also, the energy that can be expressed with the height of this wall is larger than the energy of the electron group; therefore the electron group is normally not able to climb over this wall and as a result does not migrate to the oxidizing agent. In short, it is thought that the oxidizing agent cannot be reduced.

However, the activation energy corresponding to the height of the wall may be lowered if for instance a catalyst such as an enzyme is used. As a result, the electron group included in the reducing potential water is able to migrate to the oxidizing agent rather smoothly compared to when no catalyst is used, and at the endpoint where this migration is complete, the reducing potential water is able to reduce the oxidizing agent.

In this manner, when an enzyme or similar catalyst is used, the high-energy electron group included in the reducing potential water can be more easily released, and results in the reducing power being exhibited. In other words, this is what is meant by the reducing potential water 'having latent reducing power', which may be rephrased as 'the reducing power of the reducing potential water kept under seal'. These various thought processes led to the idea that 'the key to lifting the seal on the reducing power

held by the reducing water is a catalyst.'

Now that the history of the idea of the invention has been elucidated, a synopsis of the invention will be described.

(2) Synopsis of Invention

5   (2-1) Antioxidation Method

The present invention provides an antioxidation method that includes transforming an antioxidation subject, which is in an oxidation state due to a deficiency of electrons or needs to be protected from oxidation, into a reduced state of electrons being filled, by promoting the breaking (activating) reaction of molecular hydrogen used

10   as a substrate included in hydrogen-dissolved water into a product of active hydrogen via a process employing a catalyst, which is a precious metal colloid or a hydrogen oxidation/reduction enzyme (except for those already existing in the living organism), on the hydrogen-dissolved water.

The inventors are confident that the substance that provides the negative

15   value for the ORP value of hydrogen-dissolved water such as electrolyzed water or hydrogen bubbling water is the hydrogen that is dissolved in that water. The fact that hydrogen is the ultimate reducing substance, and furthermore, the fact that hydrogen develops on the cathode side during electrolysis processing serves as proof of this conviction.

20   Nevertheless, as made clear in the history of the idea behind the invention, with the hydrogen-dissolved water as it is, the reducing power is normally kept under seal.

Therefore, in order to cast off the seal on the reducing power held by the hydrogen-dissolved water, as defined with the antioxidation method according to the

25   present invention, it has been found that the step of using a catalyst in the hydrogen-dissolved water is extremely important.

Another important factor is the existence of an antioxidation subject. If there is no antioxidation subject, then there is no stage for the antioxidation action according to the present invention to be exhibited.

30   In other words, the important factors in the present invention are 1) the hydrogen-dissolved water, 2) the catalyst, and 3) the antioxidation subject. When these three factors are organically combined, the seal on the reducing power latently held by the hydrogen is cast off to allow manifest expression of the broad antioxidation function including the reducing function. It should be noted that the expression of the

antioxidation function spoken of in the present invention is the reduced state of electrons being filled in the antioxidation subject, which is either in an oxidized state due to a deficiency of electrons or needs to be protected from oxidation. In addition, the reduced state of electrons being filled in the antioxidation subject is a concept including

5   both a case of reducing the antioxidation subject in an oxidized state, and a case of reducing an oxidizing material itself such as active oxygen species, which attempts to oxidize the antioxidation subject that needs to be protected from oxidation.

While magnitude of the reducing power here may be estimated to a certain extent through, for example, the condition of the ORP value (i.e., the stability of the

10   ORP reading or the relationship with the above-mentioned Nernst equation), ultimately it is determined depending on the effective value of the dissolved hydrogen concentration DH found using the dissolved hydrogen concentration quantitative method (described later) that uses an oxidation/reduction pigment.

Next, the technical scope that is assumed for the present invention regarding

15   these three factors will be laid out.

(2-1-1) Hydrogen Dissolved Water

Hydrogen dissolved water is assumed to be any water in which hydrogen is included. In addition, what is called water here (also referred to as raw water) includes all waters including tap water, purified water, distilled water, natural water,

20   activated charcoal processed water, ion exchange water, pure water, ultra pure water, commercially available (PET) bottled water, biological fluid (described later), and water in which molecular hydrogen is generated through a chemical reaction in the water. Furthermore, all water that includes an auxiliary agent for electrolysis or a reducing agent added to such water also falls within the technical scope of the present invention.

25   Moreover, as long as it meets the condition of being water in which hydrogen is included, it does not matter if the water is acidic, neutral, or alkaline, nor does it particularly matter if the dissolved concentration is high or low. However, since the antioxidation function expressed through application of the present invention emanates from the electrons released through the process of replacing molecular hydrogen with active

30   hydrogen through a catalyst, more significant expression of the antioxidation function may be expected with a higher dissolved concentration of molecular hydrogen.

Moreover, hydrogen-dissolved water also includes either electrolyzed water generated on the cathode side when raw water is subjected to electrolysis processing between an anode and a cathode via a membrane, or water processed through bubbling

(aeration) or pressurized filling of hydrogen into raw water. The definition is made in this way in order to make clear that electrolyzed water such as 'alkaline ion water' that is produced through existing continuous flow-type or batch electrolyzed water generation apparatus as well as hydrogen-dissolved water generated by including

5   hydrogen in raw water through external manipulation also fall within the technical scope of the present invention. Those given as hydrogen-dissolved water here are merely examples and are not intended to be limited to this. Accordingly, it should be made clear now that even if using for instance natural water and hydrogen is included therein, this does not mean that such water falls outside of the technical scope of the

10   present invention.

       However, hydrogen-dissolved water also includes reducing potential water where the ORP is a negative value, and the ORP value corresponding to the pH shows a value that is lower than the value according to the Nernst equation or ORP = -59 pH − 80 (mV). The reducing potential water mentioned here naturally includes electrolyzed

15   reducing potential water generated with the reducing potential water generation apparatus developed by the applicants herein that has: an electrolytic chamber into which raw water to be electrolyzed is supplied; at least one membrane which separates inside the electrolytic chamber from outside thereof; at least a pair of electrode plates provided inside and outside the electrolytic chamber, respectively, and sandwiches the

20   membrane; and a power supply that supplies a voltage between both electrodes, wherein the electrode plate provided inside the electrolytic chamber is given as the cathode and the electrode plate provided outside the electrolytic chamber is given as the anode; wherein the electrode plates provided outside the electrolytic chamber is provided in contact with the membrane or leaving a slight space, and it should be made

25   clear now that this also includes water that while generated with an apparatus other than such apparatus meets the conditions for reducing potential water described above. It should be now added that in the case of employing a circulating electrolysis processing technique in the reducing potential water generation apparatus wherein water that has been generated is again introduced into the electrolytic cell (electrolysis

30   chamber) so as to circulate, and then repeating this circulatory process for a predetermined length of time, as shown for instance in the following Table 1, reducing potential water may be obtained having a high dissolved-hydrogen concentration and an even lower ORP value, and superior reducing power (antioxidizing power) may be expressed with such reducing potential water.

Furthermore, it is preferable that the dissolved-hydrogen water be water in which the dissolved hydrogen concentration is greater than the saturation concentration (in terms of effective value of dissolved hydrogen concentration value found using a dissolved hydrogen concentration quantitative analysis method that uses

5   oxidation/reduction pigment) under atmospheric pressure. This is because high benchmarks of the reduction activity and antioxidation activity emanating from the antioxidant-functioning water according to the present invention can be anticipated.

Therefore, the respective physical property values of reference examples of hydrogen-dissolved water assumed by the inventors and comparative examples of water

10   in which no hydrogen is dissolved are now given. Activated charcoal processed water resulting from processing Fujisawa City tap water through an activated charcoal column, Organo purified water resulting from processing Fujisawa municipal tap water through an ion exchange column made by Organo Corporation, and an example of (PET) bottled water: 'evian' (registered trademark of S.A. des Eaux Minerales d' Evian), which

15   is supplied in Japan through Calpis Itochu Mineral Water Co., Ltd., are given as examples of subject water for purposes of comparison. A first reducing potential water subjected to continuous electrolysis processing using electrolysis conditions of a 5A constant current and flow rate of 1 L/min in the reducing potential water generation apparatus developed by the applicants herein, and a second reducing potential water

20   subjected to continuous circulating electrolysis processing for 30 minutes using the same electrolysis conditions (volume of circulatory water: 2 liters) in the same apparatus are given as examples of each type of post-processing hydrogen-dissolved water for the purpose of dissolving hydrogen in such comparative subject waters. In addition, hydrogen gas bubbling water subjected to hydrogen gas bubbling processing

25   for 30 minutes, and alkaline electrolyzed water subjected to continuous electrolysis processing using electrolysis conditions of electrolysis range '4' with a standard amount of water in a 'Mini Water' electrolyzed water generation apparatus made by MiZ Co., Ltd. are given as examples vis-à-vis each type of comparative subject water.

Furthermore, pH, oxidizing/reducing potential ORP (mV), electrical

30   conductance EC (mS/m), dissolved oxygen concentration DO (mg/L), dissolved hydrogen concentration DH (mg/L), and water temperature T (°C) are given as the various physical property values in such waters. In addition, the various types of gauges used to measure these physical property values include: a pH meter (including a temperature gauge) that is a model D-13 pH meter made by Horiba, Ltd. with a model 9620-10D

12

probe for the same; an ORP meter that is a model D-25 ORP meter made by Horiba, Ltd. with a model 9300-10D probe for the same; an EC meter that is a model D-24 EC meter made by Horiba, Ltd. with a model 9382-10D probe for the same; a DO meter that is a model D-25 DO meter made by Horiba, Ltd. with a model 9520-10D probe for the same; and a DH meter (dissolved hydrogen meter) that is a model DHD I-1 made by DKK-TOA Corporation with a model HE-5321 electrode (probe) and model DHM-F2 repeater for the same. The various physical property values of the comparative subject waters were respectively measured using these types of gauges (the same types of gauges were also used for the following measurements.)

[Table 1]

★

According to this Table 1, focusing on the dissolved hydrogen concentration (DH) measured with the dissolved hydrogen meter, with the first reducing potential water subjected to one-time electrolysis processing using the reducing potential water generation apparatus, despite the fact that the electrolyzed water was instantly removed, it was found that a high concentration of hydrogen ranging between 0.425 and 0.900 (mg/L) was dissolved therein.

In addition, in the case of the length of processing time being, for example, 30 minutes, comparing the dissolved hydrogen concentrations of the buffered electrolyzed reducing potential water (the second reducing potential water) in this reducing potential water generation apparatus and the hydrogen gas bubbling water, while the latter ranged between 0.89 and 1.090 (mg/L), the former showed that a high concentration of hydrogen ranging between 1.157 and 1.374 (mg/L) could also be dissolved therein (note that this measured data is merely for reference since performance of the reducing potential water generation apparatus is greatly improved by improving a part of the apparatus as described in working examples and reference examples described later.)

Meanwhile, it is preferable that at least one reducing agent selected from the group consisting of sulfite, thiosulfate, ascorbic acid, and ascorbate be added as required to the antioxidant-functioning water (pharmacologic-functioning water). This is because it is preferable that the dissolved oxygen concentration in the hydrogen-dissolved water be made as low as possible (as the dissolved oxygen concentration in the hydrogen-dissolved water becomes lower, preference increases, such as in the following order: 0 mg/L, 0.5 mg/L or lower, 1 mg/L or lower, 1.5 mg/L or

lower, 2 mg/L or lower, 2.5 mg/L or lower, 3.0 mg/L or lower, 3.1 mg/L or lower, 3.2 mg/L or lower, 3.3 mg/L or lower, 3.4 mg/L or lower, and 3.5 mg/L or lower) when it is necessary to prevent rapid oxidation due to the dissolved oxygen of the active hydrogen occurring through the action of the catalyst.

5   To further explain this, in hydrogen-dissolved water where a catalyst has been used, it is possible to reduce the dissolved oxygen concentration DO (mg/L) to nearly zero (mg/L) when the amount of reducing agent added is less than the chemical equivalent capable of exactly reducing the dissolved hydrogen.

As a comparative example for this, when the same amount of reducing agent

10   was added to hydrogen-dissolved water where a catalyst had not been used, significant reduction in the dissolved oxygen concentration DO (mg/L) was not achieved. This is thought to be the result of the intrinsic reducing power held by the hydrogen-dissolved water on which the seal had been lifted bringing out the reducing power held by the reducing agent more strongly.

15   Accordingly, it should be added that in the case of bottling antioxidant-functioning water (pharmacologic-functioning water) according to the present invention in the condition where both a reducing agent and a dissolved additive such as a vitamin coexist, there is also the dimension that such an additive causes the antioxidizing action and the pharmacological action intrinsically held by the additive to

20   be brought out even more strongly, and amplification activity can be expected as a result of being in an antioxidizing environment. This is because when antioxidant-functioning water (pharmacologic-functioning water) according to the present invention is bottled in the condition where both a reducing agent and the exemplary reducing ascorbic acid coexist, it means that the ascorbic acid causes the

25   antioxidizing action and the pharmacological action intrinsically held by the reducing ascorbic acid to be brought out even more strongly as a result of continuing to be in reducing form due to being in an antioxidizing environment (for details, see section 'Does antioxidant-functioning water (AOW) control oxidation of reduced vitamin C?' given later.) In this case, it is preferable to add the reducing agent such as the

30   exemplary reducing ascorbic acid in an amount greater than that required to reduce/neutralize the oxidizing material such as dissolved oxygen in the coexistent system. However, it is preferable that an appropriate amount of ascorbic acid be added in consideration of the pH expressed by the antioxidant-functioning water and the minimum recommended daily amount that should be ingested.

(2-1-2) Catalyst

The catalyst is assumed to be all those having the function of catalyzing the breaking reaction of the molecular hydrogen used as a substrate included in the hydrogen-dissolved water into a product of active hydrogen. More specifically, the essential qualities of the catalyzing function according to the present invention lies in smoothly accelerating the activation of molecular hydrogen, and within such function, accepting electrons from the molecular hydrogen (by activating one molecular hydrogen, two electrons are obtained or $H_2 \square 2e^- + 2H^+$) and donating the accepted electrons to the antioxidation subject following temporary pooling (including the idea of absorption or occlusion into the catalyst) or without pooling. It should be noted that donating the electrons to the antioxidation subject is a concept including both a case of reducing the antioxidation subject, which is in an oxidized state, and a case of reducing an oxidizing material itself such as active oxygen species that attempts to oxidize the antioxidation subject, which needs to be protected from oxidation.

The catalyst according to the present invention may be a precious metal colloid that falls within the technical scope. It should be noted that the precious metal colloid assumed with the present invention means the inclusion of platinum, palladium, rhodium, iridium, ruthenium, gold, silver, or rhenium, along with the respective salts thereof, alloy chemical compounds, or colloidal particles themselves such as complex chemical compounds, as well as mixtures of these. When making or using these precious metal colloids, reference should be made to the contents of 'Fabrication and Use of Pt Colloids (Pt koroido no tsukurikata to tsukaikata)' (NANBA, Seitaro and OKURA, Ichiro); Hyomen Kagaku (Surface Science) Vol. 21; No. 8 (1983), the contents of which are included herein by reference. In addition, the colloid mentioned in the present invention is assumed as having molecules with diameters ranging between 1 nm and 0.5 µm, which is said as showing innate behavior of a general colloid. However, when employing the exemplary Pt colloid as the precious metal colloid, it may be proper to use a molecular diameter that increases the catalytic activity of this Pt colloid, preferably ranging between 1 and 10 nm and more preferably between 2 and 6 nm. This is, as written in the above-mentioned 'Fabrication and Use of Pt colloids' by Nanba and Okura, the molecular size is derived from the trade-off relationship between the fact that the innate property is expressed as a precious metal and the fact that the surface area is increased to improve the catalytic activity. However, the colloids mentioned in the present invention are in accordance with the definition proposed by

Staudinger of Germany that 'colloids are configured with between 10^3 and 10^9 atoms.' Moreover, the precious metal colloid according to the present invention preferably has a round granular shape in order to increase the surface area. Here, since the fact that the surface area of the precious metal colloid is large means increased opportunities for 5 connection with the molecular hydrogen used as the substrate, it is superior from the viewpoint of catalytic function expressed by the precious metal colloid.

Moreover, a catalyst includes the idea of electron carriers such as a coenzyme that assists the functioning thereof, inorganic compounds, and organic compounds.

It is preferable that such an electron carrier have properties capable of 10 efficiently accepting electrons from hydrogen or a precious metal colloid, which are all electron donors, and at the same time, efficiently carrying electrons to the antioxidation subject, which is an electron acceptor. To put it more simply, the electron carrier acts to transport the hydrogen (electron).

(2-1-2-1) Candidates for the Electron Carrier

15 In the following, candidates for the electron carrier are now given. It should be noted that it does not matter if the electron carrier is oxidizing or reducing. Since the reducing electron carrier has surplus electrons beforehand, it is beneficial from the viewpoint of easily releasing electrons.

(a) Methylene blue (normally oxidizing)

20 methylthionine chloride, tetramethylthionine chloride

chemical formula = $C_{16}H_{18}ClN_3S \cdot 3(H_2O)$

Reducing methylene blue is referred to as leucomethylene blue.

(b) Pyocyanin

chemical formula = $C_{13}H_{10}N_2O$

25 One of the antibiotic substances produced by Pseudomonas aeruginosa. Pyocyanin performs reversible oxidation/reduction reactions, and there are two types of the oxidizing type: one that is alkaline and a blue color, and one that is acidic and a red color. In addition, the reducing type is colorless, as is the reduced methylene blue (leucomethylene blue).

30 (c) Phenazine methosulfate

abbreviation = PMS

chemical formula = $C_{14}H_{14}N_2O_4S$

Phenazine methosulfate tends to easily photo-decompose.

(d) 1 – Methoxy PMS

Is stable when exposed to light and was developed as a substitute for the PMS mentioned above that is unstable when exposed to light.

(e)   Chemical compounds including the iron (III) ion

Many exist such as $FeCl_3$, $Fe_2(SO_4)_3$, and $Fe(OH)_3$.   The intrinsic purpose is as

5   a reagent for obtaining Iron (III) or Fe $(^{3+})$ as an ion.   In living organisms, it is thought as existing as heme iron in the hemoglobin of red blood cells.   It should be noted that heme iron has characteristics that are different from the independent iron ion.

In particular, when acting with ascorbic acid, since it produces a hydroxyl radical ($\cdot$OH) having strong oxidizing power, the iron ion is not always required when

10   in vitro.   However, in vivo, when the iron ion coexists with nitric oxide (NO), it is said that it does not always generate the hydroxyl radical ($\cdot$OH).

In particular, although the iron (II) ion Fe $(^{2+})$ is the reduced form of the iron (III) ion Fe $(^{3+})$, there are many occasions where even with the reduced form, the oxidizing action is accentuated.   In particular, if there is lipid peroxide, a radical chain

15   reaction may easily occur.   When the iron (III) ion Fe $(^{3+})$ is reduced through ascorbic acid or the like, a radical generating chain reaction occurs if it coexists with lipid peroxide.   In other words, many lipid radicals may be produced, which may have a negative effect on living organisms.

(f)   Reduced ascorbic acid (chemical formula = $C_6H_8O_6$)

20   Exists in living organisms, but it is absorbed from outside the body, and is not synthesized by humans.

(g)   Glutathione (chemical formula = $C_{10}H_{17}N_3O_6S$)

abbreviation = GSH

Is an SH chemical compound existing in large quantities in living organisms,

25   and it is thought that humans have a gene for synthesizing this.   Glutathione is a poly-peptide configured from three amino acids (glutamic acid − cysteine − glycin = Glu-Cys-Gly), a coenzyme of glyoxylase, and is known to function as an intracellular reducing agent, an anti-aging agent, and the like.   In addition, glutathione has the function of directly (nonenzymatically) reducing oxygen (O_2).

30   (h)   Cysteine (Cys)

One of the amino acids and an SH chemical compound, it is ingested as a protein and is the final product of digestive decomposition.   Cysteine is a structural component of the above-mentioned glutathione and is an amino acid having an SH group.   As with glutathione, two cysteines (Cys) respectively release one hydrogen

atom, and become oxidized cysteine through a disulfide bond (-s-s-).

(i)   Benzoic acid ($C_7H_6O_2$)

While this rarely exists in living organisms, strawberries include approximately 0.05%. Benzoic acid is a basic reducing agent and has the function of nonenzymatically and effectively scavenging the hydroxyl radical and making it into water.

(j)   p-amino Benzoic acid ($C_7H_7NO_2$)

(k)   Gallic acid ($C_7H_6O_5$) (3,4,5-trihydroxy benzoic acid)

Widely exists in leaves, stems, and roots of plants, and is used as a general hemostatic agent and an antioxidant (preservative) in food (food additive). This alkaline solution has particularly strong reducing power. Gallic acid tends to react easily with oxygen.

It should be noted that those given as catalysts here are merely examples, and it is not intended to mean that they are limited to these. Accordingly, as long as contributing to the catalyzing reaction assumed by the present invention, it should be clearly noted that it does not mean that other parameters such as physical external forces including temperature, pressure, ultrasonic waves, or agitation may be excluded.

In addition, it should be added that the product of active hydrogen comprehensively includes atomic hydrogen (H·) and hydride ions (H·).

Moreover, catalysts such as those described here may be each used independently, or as needed, may be used in an appropriate mixture of a plurality of these. Basically, electrons are transmitted in the order of the hydrogen-dissolved water to catalyst to antioxidation subject; however, besides this, the following orders may also be possible: the hydrogen-dissolved water to electron carrier to antioxidation subject, the hydrogen-dissolved water to precious metal colloid to antioxidation subject, or the hydrogen-dissolved water to precious metal colloid to electron carrier to antioxidation subject.

(2-1-3) Antioxidation Subject

An antioxidation subject is assumed to be any subject in an oxidized state due to a deficiency in electrons or for which protection from oxidation is desired. It should be noted that oxidation mentioned here means the drawing away of electrons from a subject through the direct or indirect action of oxygen, heat, light, pH, ions, etc. In addition, to be more specific, an antioxidation subject includes for instance cells or organs of living organisms, or antioxidation substances such as vitamins, food,

unregulated drugs, medical supplies, cosmetics, animal feed, oxidation/reduction pigments (to be described later), as well as water itself, all fall within the technical scope of the present invention. It should be noted that these given as antioxidation subjects here are merely examples and it should be clearly stated here that is not

5   intended to mean that they are limited to these.

Next, the relationship between a catalyst and an antioxidation subject is described from the standpoint of the catalyst.

With the present invention, the catalyzation of the breaking reaction of the molecular hydrogen used as a substrate included in the hydrogen-dissolved water into a

10   product of active hydrogen is performed with a precious metal colloid.

The reducing potential water in which a precious metal colloid such as platinum (Pt) or palladium (Pd) colloidal particles is included (that is, precious metal colloid catalyst-added antioxidant-functioning water) is assumed here. In the case of a low alkaline reducing potential water added with Pt colloid or Pd colloid being ingested

15   through drinking, and oxidizing agents such as active oxygen species coexisting in digestion related cells (antioxidation subjects) of the living organism such as those of the intestines, these oxidizing agents are immediately reduced. In addition, when other additives such as fruit juice or vitamins (antioxidation subjects) coexist, the reducing potential water acts as the antioxidizing agent of these additives under the

20   condition of Pt colloid or Pd colloid coexisting. Such action mechanism may include the molecular hydrogen-dissolved in the reducing potential water dissociating and activating the two atomic hydrogens (H·) along and being adsorbed into the minute particle surface of the Pt colloid or Pd colloid, the formed atomic hydrogen (H·) splitting into protons and electrons in the water, and the formed electrons then being donated to

25   the antioxidation subject. Here, donating the electrons to the antioxidation subject is a concept including both a case of reducing the antioxidation subject, which is in an oxidized state, and a case of reducing an oxidizing material itself such as active oxygen species that attempts to oxidize the antioxidation subject, which needs to be protected from oxidation.

30   This sort of antioxidation function is expressed only when the three items--hydrogen-dissolved water such as the reducing potential water, the precious metal colloid used as a catalyst, and the antioxidation subject such as the digestive system cell of the living organism--come together. In other words, the reducing power is only expressed when necessary and has no operational effect when not required.

Moreover, when looking at the chemical component composition, reducing potential water, for instance, is nothing more than very ordinary water obtained by electrolyzing raw water. Accordingly, the fact that even after expressing reducing power, the water only acts as ordinary water and imparts no negative side effects onto, for example, the living organism, is especially noteworthy. To restate this in another way, the fact that the positive effects aimed for may be obtained without the any negative effects or side effects is the critical difference from conventional antioxidizing agents and radical scavenging agents.

Therefore, the antioxidant-functioning water (pharmacologic-functioning water) according to the present invention can be thought of as opening the way for pharmaceuticals/ medical supplies that prevent, improve, and treat illnesses related to/ caused by monocyte/ macrophage system cellular functions, in particular, medical conditions or malfunctioning of an organ or system and illnesses related to/caused by the increase or decrease in macrophage system cellular functions.

Specific examples of pharmaceuticals and medical products are as follows. Namely, since water generally has properties that allow it to immediately reach every location in the body including fatty membranes, cellular membranes, and the blood-brain barrier, curative effects in damaged portions may be expected by delivering the hydrogen-dissolved water (antioxidant-functioning water or pharmacologic-functioning water) in which a precious metal colloid is contained to the damaged portions of the living cells caused by activated oxygen through maneuvers such as an injection, intravenous drip, or dialysis.

The precious metal colloid catalyst here is a foreign substance to the body albeit inorganic, and when assuming this is delivered to the damaged portion of the body via a maneuver such as an injection, intravenous drip, or dialysis, there is a danger that the body's immune system will recognize this as being foreign and cause an antigen antibody reaction. In order to resolve this problem, the oral tolerance principle of the body should be clinically applied. Oral tolerance refers to the antigen-specific T/B cell non-responsiveness to a foreign antigen that enters through oral/ enteral means. Simply put, oral tolerance is the phenomena where even if a substance ingested orally is a foreign substance that may become, for example, an antigen, and if it is absorbed from the small intestine, the immune tolerance allows it. Treatment using this principle has already been tested. Accordingly, through clinical application of the principle of oral tolerance, a new door of antioxidation may be opened in clinical strategy.

(2-2) Pharmacologic-Functioning Water and Usage of the Same

The present invention provides pharmacologic-functioning water demonstrating pharmacologic function without any side effects and including antioxidant-functioning water containing hydrogen-dissolved water, which is made up of molecular hydrogen used as a substrate that is included in raw water, and a precious metal colloid, which is included in the hydrogen-dissolved water and catalyzes the breaking reaction of the molecular hydrogen into a product of atomic hydrogen, as an active principle.

Of the three important factors in the present invention, since the dissolved hydrogen water and a precious metal colloid catalyst are included in the pharmacologic-functioning water employing this constitution, when put in contact with the antioxidation subject, the seal on the reducing power latently held by the hydrogen is cast off to allow expression of the antioxidation function and pharmacologic function specific to the present invention. It should be noted that the antioxidation function, which transforms an antioxidation subject into a reduced state of electrons being filled, is a concept including both a case of reducing the antioxidation subject, which is in an oxidized state, and a case of reducing an oxidizing material itself such as active oxygen species that attempts to oxidize the antioxidation subject, which needs to be protected from oxidation.

However, in the case of pharmacologic-functioning water that adopts the aforementioned constitution being ingested through drinking, and for instance, the large intestine being the antioxidation subject, there is a problem where it is impossible to achieve the primary objective since almost all of latent reducing power of the hydrogen is unsealed before reaching the large intestine.

Therefore, it is preferable that processing or manipulation be employed on the precious metal colloid used as a catalyst in order to adjust the activation time and/or the reaction time of the catalyst.

Here, the processing or manipulation for adjusting the activation time and/or the reaction time of the catalyst is, as shown in FIG. 3 or FIG. 4, processing to seal an active principle factor of a precious metal colloid or a combination of a precious metal colloid and ascorbic acid (AsA) in an enteric capsule or the like falling within the technical scope of the present invention, with the aim of having the primary catalytic action begin when the active principle factor reaches the subject portion such as the large intestine or small intestine.

Furthermore, the present invention provides a living organism-applicable fluid using the pharmacologic-functioning water as an active principle, and is prepared so as to allow usage on living organisms including drinking, injection, intravenous drip, dialysis, external use (application to skin or mucous membrane), skin care, and cosmetics.

Namely, using the pharmacologic-functioning water according to the present invention on living organisms for intended applications such as injection, intravenous drip, and dialysis, in particular, flowing through the bloodstream as is, adjusting the osmotic pressure so as to be almost isotonic with blood and adjusting the pH so as to lie within a physiologic range of liquid (e.g., pH 4.5 through 8.0, preferably, pH 7.0 through 7.8, more preferably pH 7.1 through 7.5) for the pharmacologic-functioning water according to the present invention must be implemented. In this case, the osmotic pressure-adjusting substance is not particularly limited so long as it can be physiologically tolerable. For example, various electrolytes (e.g., dissolved salts of inorganic elements such as sodium, potassium, calcium, magnesium, zinc, iron, copper, manganese, iodine, and phosphorus), saccharides such as glucose and hyaluronic acid, proteins such as albumin, and amino acids may be used. In addition, the pH adjustor is not particularly limited so long as it can be physiologically tolerable. For example, various organic acids, mineral acids, organic bases, and mineral bases may be used. In particular, organic acids are preferably used. For example, citric acid, acetic acid, succinic acid, gluconic acid, lactic acid, malic acid, maleic acid, molonic acid, and the like can be used as the organic acid, and hydrochloric acid, phosphoric acid, and the like can be used as the mineral acid. On the other hand, sodium citrate, sodium gluconate, sodium lactate, sodium malate, sodium acetate, sodium maleate, sodium malonate, and the like can be used as the organic base, and alkali hydroxide metal and the like can be used as the mineral base.

Furthermore, in order to improve symptoms of a living organism, more specifically a human, it is preferable to use transfusion fluid prepared by mixing arbitrary components of the pharmacologic-functioning water (antioxidant-functioning water) according to the present invention including chemical components such as various electrolytes, amino acids, high calorie components, enteral feeding products, and pharmaceutical components such as vitamins and antibiotics. It should be noted that dissolved salts of the inorganic elements such as sodium, potassium, calcium, magnesium, zinc, iron, copper, manganese, iodine, phosphorus, and the like can be used

as the various electrolytes. In addition, essential amino acids, non-essential amino acids, and/or salts of these amino acids, esters, N-acyl substances, or the like can be used as the amino acid. Furthermore, monosaccharides such as glucose, fructose, and the like, and disaccharides such as maltose and the like can be used as the high calorie

5 components.

Here, it may be thought that with the living organism-applicable fluid, which is the pharmacologic-functioning water (antioxidant-functioning water) according to the present invention for many applications including drinking, injection, intravenous drip, dialysis, external use (application to skin or mucous membrane), skin care, and

10 cosmetics, in which vitamins such as vitamin C and amino acid (superior protein) are included, immune system activation for living organisms in conformity with the following working-action mechanism can be expected. Namely, vitamins such as vitamin C have radical scavenging activity as with the pharmacologic-functioning water (antioxidant-functioning water) of the present invention, and act as a coenzyme for

15 synthesizing or demonstrating primary functions of living-organism enzymes (such as SOD, catalase, glutaminperoxidase, and interferon synthesized enzyme), which controls the metabolism of living organisms, and interferon (a substance made of saccharum and protein, demonstrating immunity). In addition, amino acids (superior protein) play an important role of a raw material for living-organism enzymes and interferon.

20 Here, it is assumed that excessive amounts of ($\cdot O_2^-$) generate locally in living organisms. As a result, vitamins such as vitamin C and vitamin E and living-organism enzymes such as SOD, catalase, and glutathionperoxidase collaborate and scavenge ($\cdot O_2^-$). At this time, since vitamins such as vitamin C and vitamin E are self-oxidized in the process of reducing/ scavenging ($\cdot O_2^-$), they cannot perform their primary duties

25 including the role as a coenzyme. As a result, primary functions or synthesis of living-organism enzymes and interferon in living organisms decreases, thereby leading to a decrease in immunity.

Meanwhile, it may be thought that with the living organism-applicable fluid, which is pharmacologic-functioning water (antioxidant-functioning water) according to

30 the present invention in which vitamins such as vitamin C and amino acid (superior protein) are included, immune system activation of living organisms can be expected as a result of promoting synthesis or primary functions of the living-organism enzymes and interferon, caused by supplying amino acids and devotion to the primary duties including acting as a vitamin coenzyme, along with scavenging of ($\cdot O_2^-$) by the

pharmacologic-functioning water.

It should be noted that even with pharmacologic-functioning water (antioxidant-functioning water) according to the present invention not containing vitamins and amino acids, living organism-applicable fluid according to the present invention containing vitamins, or living organism-applicable fluid according to the present invention containing amino acids, it may be thought that immune system activation of living organisms in conformity with the exemplary working-action mechanism described above can be expected since consumption of vitamins existing in the living organisms can be controlled, and vitamins can devote to their primary functions including acting as a coenzyme.

Meanwhile, it is essential that safety be guaranteed when using a precious metal colloid as a catalyst for application in a living organism. More specifically, it is necessary to consider biocompatibility including the acute toxicity of the precious metal colloid itself. However, there should not be much problem with biocompatibility because with for example platinum and palladium, even when it is ingested by a person nearly all of it may pass through the liver and be promptly eliminated in urine, and in addition, it has been allowed as a food additive by the Japanese Ministry of Health, Labor, and Welfare (there are no restrictions on the amount of additives). One more important point to be taken into account is that it is preferable to include some sort of dispersion agent in order for the precious metal colloid to disperse into the antioxidant-functioning water stably and evenly. For instance, in the case where it will be ingested through drinking or used for skin care or as cosmetics, that which has dispersion agent function should be appropriately selected from those allowed by the Japanese Ministry of Health, Labor, and Welfare as food additives. In this case, the exemplary polyvinyl pyrrolidone (PVP), gelatin, and sucrose esters of fatty acids, which are hypoallergenic and widely used in cosmetics and medical products, may be favorably used. It should be noted that including sucrose esters of fatty acids, polyvinyl pyrrolidone (PVP), gelatin, for example, in antioxidant-functioning water as a component for forming a dispersion agent or a protective membrane (has a catalytic activity-adjusting function) for a precious metal colloid is included in the category of processing or manipulation for adjusting the catalytic activity time period and/or reaction time period in relation to the appended Claims.

Such antioxidant-functioning water (pharmacologic-functioning water) may be applied to, for example, the following industrial fields.

Firstly, application may be made in the fields of medicine and pharmaceuticals. For example, it may be used as base water in the manufacturing process of transfusion fluid and other medical agents. In addition, it may also be used as artificial dialysis fluid, peritoneal dialysis fluid, and a therapeutic agent for diseases. Furthermore, it

5   may also be favorably used as preservation fluid for transplanted organs when transplanting living organs (in this case, it is preferable that osmotic regulation is performed separately.) Through this, it is possible to expect prevention/ treatment of various diseases caused by active oxygen species, inhibition of aging and reduction of side effects due to pharmaceuticals, and improvements in preservation quality of

10 transplanted organs.

Secondly, application may be made as a prevention/ treatment agent for aging and degeneration caused by oxidation of cutaneous tissue. For example, it may be used in the manufacturing process of cosmetic toners and other cosmetic products.

Thirdly, application may be made in antioxidant food, functional food, and

15 health food. For example, it may be used in food manufacturing processes.

Fourthly, application may be made in potable water, processed water, and the like. For example, it may be used as drinking water (antioxidant water) and healthy drinks, and also for use as base water in processed potable water such as canned juices, canned coffees, (PET) bottled water, and soft drinks.

20 Fifthly, application may be made to reduce contamination/deterioration of food due to fertilizers, herbicides, pesticides, etc., and also maintain freshness. For example, it may be used as a pre-shipment rinsing fluid for vegetables, fruits, and the like.

Sixthly, application may be made as a substitute for antioxidants,

25 anti-deterioration agents, anti-decomposition agents, anti-contamination agents, deodorants, and freshness-keeping agents, and the like in prepared food manufacturing. More specifically, it may be used for instance as a substitute for the over 347 types of food additives.

To further explain this, it has been pointed out that the involvement of radicals

30 including active oxygen species in oxidation, aging, deterioration of quality, decomposition, contamination, deodorization, and loss of freshness is one of the important factors of a mechanism which causes expression and deterioration thereof. As a result, radicals including active oxygen species may lead to serious damage, such as health problems, expression of diseases, deterioration of physiological functions,

degeneration of cosmetic appearance, decrease in commercial value, decrease in productivity, and increase of living/ natural environmental burdens. This also causes industrial losses such as increase in medical costs due to diseases easily occurring but difficult to remedy, opportunity loss, and high cost expenses in production and distribution. There are limited cases where the involvement of the active oxygen species is desirable, for example, in sterilization, disinfection, bleaching, and the like. In most cases, the active oxygen species only have negative influences. The present invention provides a most effective, low cost and widely applicable means for solving the various industrial problems due to the radicals including the above-mentioned active oxygen species.

Cases of suddenly exposing a living organism to large amounts of the active oxygen species are relatively limited to, for example, emergency blood recirculation during operations, organ transplants, burns on the entire body, and pulmonary emphysema due to sudden surfacing when diving. In most cases, a small amount of active oxygen species causes injury, leading to extensive harmful effects over time.

There are various occasions where oxygen and oxygen molecule-containing chemical compounds in normal or inert condition are activated and even changed to radicals, for example, with the metabolizing process and accumulation of waste products in living organisms; when being exposed to direct sunlight, ultraviolet rays, or radiation; when touching carcinogenic substances, mutagenic compounds, or heavy metals; when suffering from a burn or viral infection; or when suffering from a cut or cytoclasis. In addition, when exposed to food, drink or feed in the nature of the active oxygen species, cigarette smoke, smoke and exhaust fumes, or chlorinated organic solvents, not only being directly effected by the radical action from these elements, but also generation of active oxygen species is caused or induced in the living organisms. Although the amount of generated active oxygen species is extremely small and localized in the beginning, as the condition of generation thereof surpassing the normal level continues, or the condition of the generated active oxygen species not being completely removed and still remaining continues, the amount of active oxygen species gradually increases at a certain accelerating rate, and adverse effects as with the above-mentioned cases begin to express.

In other words, the active oxygen species cause damage in a different way than from enzyme reactions and normal chemical reactions (non-radical reactions) such as the oxidation/ reduction reaction where its generation and influence increase

continuously. In short, deterioration and degeneration of physiological functions inherently progress due to the generation of active oxygen species, and it rapidly comes to be expressed as a severe injury as if it suddenly becomes manifest one day.

To exemplify cases of human diseases, it is said that chronic oxidation stress burden due to active oxygen species is the principal cause that is involved in the underlying mechanism of various diseases such as diabetes (particularly insulin-independent diabetes), liver cirrhosis (particularly fatty liver cirrhosis), cardiovascular diseases (particularly arteriosclerosis) including angina pectoris, dementia (particularly cerebral infarction), and malignant tumors (mainly chemical carcinogenesis). In addition, deterioration of physiological functions due to aging or exhaustion of physical strength, abnormal exasperation of metabolism and breathing due to continuous strenuous exercise, excess and deficiency of nutritional balance and intake, and insufficient sleep and exercise are expressed as a phenomenon such as aging, degeneration, or fatigue. In the above-mentioned phenomena, metabolic waste products become easily accumulated, and it is confirmed that the generation of active oxygen species or amount of residual accumulation thereof increases along with reconstruction failure and function deterioration of cellular tissue or skin. It is an important factor in aggravating symptoms or delaying recovery thereof.

To exemplify cases of animals and plant other than humans, excessive generation or residual accumulation or exposure of active oxygen species causes various health impairments or growth disorders as with the case of humans; as a result thereof, this also leads to reduction in productivity and commercial value such as decrease in milking, deterioration of carhosity and decline in fattening rate, decline in oviposition rate, poor breeding including depilation, decline in breeding success rate, increase suffering from insect plague, decrease in appreciative value, decrease in cultivated harvest and catch, and deterioration of quality thereof in industries, such as breeding of domestic animals, breeding of pets, aquaculture, and plant culture.

To exemplify cases of food, drink and animal feed, generation of active oxygen species due to exposure to ultraviolet rays, mixing of raw materials effected by active oxygen species, and residual hydrogen peroxide, which is a raw material of active oxygen species and is used for disinfection and bleaching during the manufacturing process thereof, induce oxidizing destruction of nutritional elements and effective elements such as vitamins, decomposing fats and emulsion breakdown with fat-containing or -using elements, color fading or discoloration in pigment-containing or

-using elements, and deterioration from cuts with perishable products, which are accompanied by fetor and degrading taste and quality of food that develop at an accelerating pace, leading to significant deterioration of quality.

To exemplify an environmental case, existence of suspended matters affected by or easily affected by radicals including active oxygen species provides an environment where fetor, allergy, or inflammation occurs easily in a living space or working place, and promotes change in water quality.

It is safe to say that damages in the above-mentioned cases may develop even if the active oxygen species are not involved, and development thereof is drastically promoted when the active oxygen species are involved.

When radicals including active oxygen species are involved in increasing damages, it is difficult to prevent or suppress the damages with the conventional antioxidants, anti-aging agents, anti-deterioration agents, anti-decomposition agents, anti-contamination agents, deodorants, and freshness-keeping agents. The inventors are highly confident that the only solution thereof is the present invention of the antioxidation method, antioxidant-functioning water, and usage of the same.

Since the present invention has a principal action of antioxidation and never produces side effects associated therewith compared to the usage of an active oxygen species scavenger such as the conventional antioxidant, high benchmarks of both of safety and radical scavenging efficiency can be simultaneously achieved, and many problems such as degradation of flavor or color tone, breakdown of physical properties, higher costs, fear of secondary infection, difficult to use, and limited application that were difficult to solve with conventional materials can be resolved. In addition, since the present invention is not easily affected by pH, as described in the following embodiments, it can be widely applied in the fluid field from acidity to alkalinity differing from the case of enzymes or antioxidants, and by demonstrating favorable effects at room temperature, it proves extremely industrially useful in broad fields such as food and pharmaceutical fields.

(OPERATION AND EFFECTS OF THE INVENTION)

As described above, the important factors in the present invention are 1) the hydrogen-dissolved water, 2) the precious metal colloid catalyst, and 3) the antioxidation subject. When these three factors are organically combined, the seal on the reducing power latently held by the hydrogen is cast off to allow manifest expression of the antioxidation function/ pharmacologic function.

The pharmacologic-functioning water according to the present invention can demonstrate pharmacologic function without any side effects and including antioxidant-functioning water as an active principle containing hydrogen-dissolved water, which is made up of molecular hydrogen used as a substrate that is included in raw water, and a precious metal colloid, which is included in the hydrogen-dissolved water and catalyzes the breaking reaction of the molecular hydrogen into a product of atomic hydrogen.

Namely, the present invention of the pharmacologic-functioning water and usage of the same demonstrates excellent radical scavenging functions in a wide pH range from acidity to alkalinity even at room temperature.

Therefore, the usage of the present invention is expected in order to prevent or improve various disturbances such as oxidizing stress or aging, which is easily caused by involvement of the active oxygen species during physiological activity in an organism such as humans, animals other than humans, plants, zymotic microorganism, and cultured cells, or in a field where prevention of reduction of activity and deterioration of quality such as transformation, deconstruction, decomposition, contamination, off-odor, loss of freshness, reduced effectiveness, and deterioration of efficiency of products in breeding, aquaculture, cultivation, fermentation, incubation, fabrication, and preservation, or in the production process thereof are in demand.

Furthermore, the availability of the present invention is expected in various industrial fields such as food products, animal feed, pharmaceutical/medical supplies, unregulated drugs, cosmetics, cleaning agents, deodorants, sanitary goods, garments, materials for maintaining freshness, packaging containers, animal breeding, aquaculture, plant cultivation, fermentation, and incubation.

Some exemplary specific aspects, which can be provided to a user to easily exhibit the availability of the present invention and easy utilization thereof, of the groups of products in the above-mentioned industries are as follows.

For example, in the field of food products, the present invention can be utilized as a food additive having an advanced active oxygen species-removing function for improving the quality of processed food products and keeping quality, or maintaining freshness of perishable food products, or for maintaining health and preventing diseases by providing specified health food and healthy food products containing the pharmacologic-functioning water of the present invention as an active principle, respectively. In the field of animal feed, the present invention can be utilized as

additive for animal feed or pet food for managing health, and improving animal feed efficiency and productivity.

In the fields of medical supplies, unregulated drugs, cosmetics, and medical tools provided in each sub-section in Section 2 of the Japanese Drugs, Cosmetics and Medical Instruments Act, not only is the present invention useful for improving the quality of preparation as pharmaceutical additives and cosmetic additives, but it can also be used as an active principal for treating and preventing diseases, improving physical conditions and constitution, maintaining and improving beauty, and preserving sanitary conditions and comfortable environments. Of these, in the field of medical supplies, the availability of the present invention is particularly expected in medical treatment water for diseases, which may express, take a delayed recovery time, or aggravate due to involvement of the active oxygen species, or in general, fields such as medicines for revitalizing health, digestive medicines, cold medicines, medicines for stomtic/ rhinitics, eye drops, and dermatologic medicines. As for unregulated drugs, the present invention is highly available in the fields of medicated dental agents, mouth refrigerant, medicated cosmetics, hair agents, bath agents, underarm deodorants, and sanitary treatment products. Furthermore, in the case of cosmetics, it is highly available in the fields of hair care products, shampoo cosmetics, skin toners, skin creams and milky lotions, facial masks, foundation, lip rouge, facial wash, soaps, and dental agents.

It should be added that the pharmacologic-functioning water according to the present invention can be provided in various specific aspects, and therefore the embodiments and availability of the present invention are not limited to the above-mentioned exemplary cases.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph showing the Nernst equation;

FIG. 2 is a diagram for describing the conditions of an illumination test using an LED;

FIG. 3 is a diagram for describing an exemplary application of the present invention;

FIG. 4 is a diagram for describing an exemplary application of the present invention;

FIG. 5 is a vertical cross-sectional view showing the basic configuration of a reducing potential water generation apparatus 11 used for producing base water

(hydrogen-dissolved water) in antioxidant-functioning water according to the present invention;

FIG. 6 is a diagram showing reduction activity evaluation test results for Pt colloid catalyst-added electrolyzed water using methylene blue color change;

FIG. 7 is a diagram showing reduction activity evaluation test results for Pt colloid catalyst-added electrolyzed water using methylene blue color change;

FIG. 8 is a diagram showing reduction activity evaluation test results for Pt colloid catalyst-added hydrogen-dissolved water using methylene blue color change;

FIG. 9 is a diagram showing reduction activity evaluation test results for Pt colloid catalyst-added hydrogen-dissolved water using methylene blue color change;

FIG. 10 is a diagram showing reduction activity evaluation test results for Pd colloid catalyst-added hydrogen-dissolved water using methylene blue color change;

FIG. 11 is a diagram showing reduction activity evaluation test results for Pd colloid catalyst-added hydrogen-dissolved water using methylene blue color change;

FIG. 12 is a diagram showing reduction activity evaluation test results for mixed precious metal (Pt + Pd) colloid catalyst-added hydrogen-dissolved water using methylene blue color change;

FIG. 13 is a diagram showing reduction activity evaluation test results for mixed precious metal (Pt + Pd) colloid catalyst-added hydrogen-dissolved water using methylene blue color change;

FIG. 14 is a diagram showing reduction activity evaluation test results for Pt colloid catalyst-added electrolyzed water (pre-electrolysis processing addition vs. post-electrolysis processing addition) using methylene blue color change;

FIG. 15 is a diagram showing antioxidation activity evaluation test results for Pt colloid catalyst-added electrolyzed water using DPPH radical color change;

FIG. 16 is a diagram showing antioxidation activity evaluation test results for Pt colloid catalyst-added electrolyzed water using DPPH radical color change;

FIG. 17 is a diagram showing antioxidation activity evaluation test results for catalyst-added hydrogen-dissolved water (degasification treatment + hydrogen gas inclusion treatment) using DPPH radical color change;

FIG. 18 is a diagram showing antioxidation activity evaluation test results for catalyst-added hydrogen-dissolved water (degasification treatment + hydrogen gas inclusion treatment) using DPPH radical color change;

FIG. 19 is a diagram showing reduction activity evaluation test results for

enzyme hydrogenase catalyst-added hydrogen-dissolved water (degasification treatment + hydrogen gas inclusion treatment) using methylene blue color change;

FIG. 20 is a diagram showing reduction activity evaluation test results for enzyme hydrogenase catalyst-added hydrogen-dissolved water (degasification treatment + hydrogen gas inclusion treatment) using methylene blue color change;

FIG. 21 is a diagram for describing a method for quantitative analysis of dissolved hydrogen concentration through redox titration with oxidation/reduction pigment;

FIG. 22 is a diagram for describing a method for quantitative analysis of dissolved hydrogen concentration through redox titration with oxidation/reduction pigment;

. FIG. 23 is a diagram for describing the comparison of the measured value and the effective value of the concentration of dissolved hydrogen DH in each type of sample water;

FIG. 24 is a diagram for describing a cytochrome (c) reduction method;

FIG. 25 is a diagram for describing an epinephrine oxidation method;

FIG. 26 is a diagram showing characteristics of the radical scavenging activity expressed in Pt colloid catalyst-containing hydrogen-dissolved water changing over time using the Pt colloid concentration as a main parameter;

FIG. 27 is a diagram showing characteristics of the radical scavenging activity expressed in Pd colloid catalyst-containing hydrogen-dissolved water changing over time using the Pd colloid concentration as a main parameter;

FIG. 28 is a diagram showing characteristics of the radical scavenging activity expressed in Pt colloid catalyst-containing hydrogen-dissolved water changing over time using the Pt colloid concentration as a main parameter;

FIG. 29 is a diagram showing characteristics of the radical scavenging activity expressed in mixed (Pt + Pd) colloid catalyst-containing hydrogen-dissolved water changing over time using the mixed (Pt + Pd) colloid concentration as a main parameter;

FIG. 30 is a diagram showing characteristics of the radical scavenging activity expressed in mixed (Pt + Pd) colloid catalyst-containing hydrogen-dissolved water changing over time using the mixed (Pt + Pd) colloid concentration as a main parameter and a mixed mole ratio Pt : Pd as a sub parameter;

FIG. 31 is a diagram showing characteristics of the radical scavenging activity

expressed in mixed (Pt + Pd) colloid catalyst-containing hydrogen-dissolved water changing over time using the mixed (Pt + Pd) colloid concentration as a main parameter and a mixed mole ratio Pt : Pd as a sub parameter;

FIG. 32 is a diagram showing characteristics of the radical scavenging activity expressed in pre-electrolysis Pt colloid catalyst-added one-pass electrolyzed water changing over time using Pt colloid concentration as a main parameter;

FIG. 33 is a diagram showing characteristics of the radical scavenging activity expressed in pre-electrolysis Pd colloid catalyst-added one-pass electrolyzed water changing over time using Pd colloid concentration as a main parameter;

FIG. 34 is a diagram showing characteristics of the radical scavenging activity expressed in pre-electrolysis Pt colloid catalyst-added circulating electrolyzed water changing over time using the Pt colloid concentration as a main parameter;

FIG. 35 is a diagram showing characteristics of the radical scavenging activity expressed in pre-electrolysis Pd colloid catalyst-added circulating electrolyzed water changing over time using the Pd colloid concentration as a main parameter;

FIG. 36 is a diagram showing characteristics of the radical scavenging activity expressed in an AsA solution changing over time using the AsA solution concentration as a main parameter;

FIG. 37 is a diagram showing characteristics of the radical scavenging activity expressed in catalyst-added hydrogen-dissolved water changing over time using differences in precious metal catalyst (fixed concentration) type as a main parameter;

FIG. 38 is a diagram showing a working-action mechanism of the Pt colloid catalyst in a hydrogen-oxygen coexisting solution system;

FIG. 39 is a diagram showing a working-action mechanism of the Pd colloid catalyst in the hydrogen-oxygen coexisting solution system;

FIG. 40 is a diagram showing effective values of the dissolved-hydrogen concentration DH according to working examples;

FIG. 41 is a diagram showing influences of Pt colloid catalyst-containing two-time electrolyzed water (AOW) on the life span of nematode C. elegans;

FIG. 42 is a diagram showing influences of Pt colloid catalyst-containing two-time electrolyzed water (AOW) on the life span of nematode C. elegans;

FIG. 43 is a diagram showing characteristics of reduced vitamin C residual ratio (%) changing over time when reduced vitamin C is included in various neutral test waters using a buffer solution (pH 7.4);

FIG. 44 is a diagram showing characteristics of reduced vitamin C residual ratio (%) changing over time when reduced vitamin C is included in various basic test waters using a buffer solution (pH 9.0);

FIG. 45 is a diagram showing characteristics of reduced vitamin C residual ratio (%) changing over time when reduced vitamin C is included in various acidic test waters using a buffer solution (pH 2.2);

FIG. 46 is a diagram showing influences of consumption of precious metal colloid catalyst (Pt or Pd) -containing electrolyzed water (AOW) on control of oxidation damage on genetic DNA;

FIG. 47 is a diagram showing influences of consumption of precious metal colloid catalyst (Pt or Pd) -containing electrolyzed water (AOW) on control of lipoperoxidation;

FIG. 48 is a diagram showing influences of precious metal colloid catalyst-containing electrolyzed water (AOW) on weight shift in rats;

FIG. 49 is a diagram showing influences of precious metal colloid catalyst-containing electrolyzed water (AOW) on arthritis score transition;

FIG. 50 is a diagram showing influences of precious metal colloid catalyst-containing electrolyzed water (AOW) on sensitized limb volume transition; and

FIG. 51 is a diagram showing effective values of the dissolved-hydrogen concentration DH expressed in antioxidant-functioning water (pharmacologic-functioning water) used for various test groups in a pharmacological test.

BEST MODE FOR CARRYING OUT THE INVENTION

An exemplary embodiment of the present invention is described in detail forthwith while referencing the drawings.

To begin with, referencing FIG. 5, the basic structure of a reducing potential water generation apparatus 11 according to the present invention used for producing base water (hydrogen-dissolved water) in antioxidant-functioning water (pharmacologic-functioning water) is described.

The reducing potential water generation apparatus 11 of this embodiment is formed with an inlet 111 for conducting raw water such as pure water, an outlet 112 for extracting the generated reducing potential water, and an electrolysis chamber 113 between the inlet 111 and the outlet 112. Although not limited to the following configuration, the reducing potential water generation apparatus 11 of this

embodiment has the inlet 111 formed at the bottom of a casing 114 so as to allow conduction of raw water in a direction that is substantially perpendicular to the surface of the paper on which the drawing is shown. The outlet 112 is formed in the top portion of the casing 114 so as to allow intake of the electrolyzed water in a direction that is substantially perpendicular to the surface of the paper on which the drawing is shown.

In addition, a porous membrane 115 is provided on both the left and right inner walls of the reducing potential water generation apparatus 11, and an electrode plate 116 is provided outside each of these respective membranes 115. The other electrode plates 117 are provided inside the electrolysis chamber 113 with the respective principal surfaces thereof facing a corresponding electrode plate 116.

Thus there are two pairs of electrode plates facing each other, each having a membrane 115 sandwiched therebetween. These two pairs of electrode plates 116 and 117 are connected to a direct-current power source (power supply) 12; wherein an anode thereof is coupled to one of the plates in each pair of electrode plates 116 and 117, and a cathode thereof is coupled to the other electrode plate. When generating reducing potential water in the electrolysis chamber 113, for example as shown in FIG. 5, the cathodes of the direct-current power source 12 are connected to the electrode plates 117 arranged inside the electrolysis chamber 113, and the anodes are connected to the electrode plates 116 arranged outside the electrolysis chamber 113.

It should be noted that in the case of generating electrolyzed oxidation water in the electrolysis chamber 113, the anodes of the direct-current power source 12 may be connected to the electrode plates 117 arranged inside the electrolysis chamber 113, and the cathodes may be connected to the electrode plates 116 arranged outside the electrolysis chamber 113.

The electrode plates 116 and 117 used in this example are configured through baking and coating across the entire titanium material surface one or more combinations of precious metals selected from a group consisting of platinum, iridium, palladium, and the like. Furthermore, the electrode plates 116 and 117 have multiple punched holes as described later.

It is preferable that the membrane 115 used in this embodiment have properties that allow easy permeation of water flowing through the electrolysis chamber 113 yet allow little permeated water to leak out. More specifically, with the reducing potential water generation apparatus 11 of this embodiment, during

electrolysis the membrane 115 itself and the narrow space S between the membrane 115 and the electrode plate 116 forms a water screen, and electric current flows into both of the electrode plates 116 and 117 via this water screen. Accordingly, the water configuring this water screen is successively replaced, which becomes important since

5 it increases the effectiveness of the electrolysis. In addition, if the water that permeates the membrane 115 leaks out from between the membrane 115 and the electrode plate 116, processing thereof becomes necessary, and therefore it is preferable that the membrane have water-holding properties strong enough to keep the permeated water from dripping down. However, when employing a solid

10 electrolyte film, for example, as the membrane, since this solid electrolyte film itself has electrical conduction properties, the narrow space S formed between the membrane 115 and the electrode plate 116 may be omitted.

An exemplary membrane 115 may include a nonwoven polyester fabric or a polyethylene screen, and the film material may be a chlorinated ethylene or a

15 polyfluorinated vinylidene and a titanium oxide or a polyvinyl chloride, and be a solid electrolyte film or a porous film having a thickness ranging between 0.1 and 0.3 mm, an average pore diameter ranging between 0.05 and 1.0 μm, and a permeable water rate that is no greater than 1.0 cc/cm^2·min. If a cation exchange membrane is to be utilized for the membrane 115, then a cation exchange group perfluorosufonic acid film

20 having a base material of polytetrafluoroethylene (e.g., the Nafion(R) Membrane made by DuPont(tm)), a copolymer consisting of a cation exchange group vinyl ether and tetrafluoroethylene (e.g., flemion film made by Asahi Glass Co.), or the like may be used.

Meanwhile, the distance between the respective pairs of mutually facing

25 electrode plates 116 and 117 sandwiching such membranes 115 may range between 0 mm and 5.0 mm, and is more preferably 1.5 mm. Here, a distance of 0 mm between the electrode plates 116 and 117 denotes the exemplary case of using a zero gap electrode wherein electrode films are formed directly on both principal surfaces of the respective membranes 115, and means that there is a distance substantially equal to

30 the thickness of a membrane 115. It is also allowable to use zero gap electrodes where an electrode is formed on only one of the principal surfaces of the respective membranes 115. In addition, in the case where such a zero gap electrode is employed, it is preferable that openings (e.g., punched holes) or spaces be provided for electrode plates 116 and 117 to allow the gas that develops from the electrode surface to be

released to the back surface opposite the membrane 115.   It should be noted that the configuration providing such openings or spaces in the electrode plates 116 and 117 may also be employed for the electrode plates arranged in the electrolysis tank shown in FIG. 5.

5   In addition, the distance between the electrode plates 117 and 117, while not specifically limited, may range between 0.5 mm and 5 mm, and more preferably is 1 mm.

In order to generate reducing potential water using the reducing potential water generation apparatus 11 with such configuration, to begin with, the negative

10   electrodes (-) of the direct-current power source 12 are connected to the two electrode plates 117 and 117 arranged inside the electrolysis chamber 113, the positive electrodes (+) of the direct-current power source 12 are connected to the electrode plates 116 and 116 arranged outside the electrolysis chamber 113, and voltage is applied to the two pairs of mutually facing electrode plates 116 and 117 sandwiching

15   the respective membranes 115.   As pure water etc. is supplied from the inlet 111, electrolysis of water is performed in the electrolysis chamber 113, wherein the following reaction is occurring at the surface of the electrode plates 117 and in the vicinity thereof:

$$2H_2O + 2e^- \square\ 2OH^- + H_2 \square$$

20   Moreover, at the surface of the electrode plates 116 outside the electrolysis chamber 113 sandwiching the respective membranes 115, in other words between each electrode plate 116 and each membrane 115, the following reaction is occurring:

$$H_2O - 2e^- \square\ 2H^+ + 1/2 \cdot O_2 \square$$

As this H^+ ion permeates the membrane 115 and passes through, a part

25   thereof accepts an electron e^- from the cathode plate 117 to become hydrogen gas dissolved in the generated electrolyzed water on the cathode side.   This causes the electrolyzed water generated on the cathode side (i.e., inside the electrolysis chamber 113) to become reducing potential water having a lower oxidation/reduction potential (ORP) than electrolyzed water generated using conventional membrane electrolysis

30   technology.

In addition, since the remainder of the H^+ ion passed through the membrane 115 reacts with the OH$^-$ ion in the electrolysis chamber 113 and reverts to water, the pH of the reducing potential water generated with the electrolysis chamber 113 changes slightly towards neutrality.   In other words, reducing potential water having

a pH that is not very high yet having a low ORP is obtained. The reducing potential water including the hydroxide ion generated in this manner is supplied from the outlet 112.

It should be noted that when wanting to make the reducing potential water obtained through such electrolysis processing a certain desired pH level, the pH level of the raw water may be adjusted beforehand using a pH buffer acting salt solution such as phthalate, phosphate, or borate. This is because the pH of the raw water is not changed much with this reducing potential water generation apparatus 11. More specifically, for instance, if a pH that tends towards alkalinity is wanted for intended applications such as rinsing silicon wafers or drinking, the pH level of the raw water may be managed and adjusted to approach alkalinity. If a pH that is substantially neutral for intended applications such as drinking, injection solution, intravenous drip solution, or dialysis fluid, the pH level of the raw water may be adjusted to be substantially neutral. Moreover, if a pH that is slightly acidic for intended applications such as cosmetics, the pH level of the raw water may be adjusted to approach slightly acidic levels.

While that shown in FIG. 5 has been described as an apparatus that generates reducing potential water in the embodiment described above, this apparatus 11 is also applicable to cases where oxidizing potential water is produced. In this case, the positive electrodes (+) of the direct-current power source 12 may be connected to the two electrode plates 117 and 117 arranged inside the electrolysis chamber 113, and the negative electrodes (-) of the direct-current power source 12 connected to the electrode plates 116 and 116 arranged outside the electrolysis chamber 113, to apply voltage to the two pairs of mutually facing electrode plates 116 and 117 sandwiching the respective membranes 115.

As pure water or the like is supplied from the inlet 111, electrolysis of the water is performed in the electrolysis chamber 113, wherein the following reaction is occurring at the surface of the electrode plates 117 and in the vicinity thereof:

$$H_2O -2e^- \square 2H^+ + 1/2 \cdot O_2 \square$$

Meanwhile, at the surface of the electrode plates 116 outside the electrolysis chamber 113 sandwiching the respective membranes 115, namely at the water screen between each electrode plate 116 and each membrane 115, the following reaction is occurring:

$$2H_2O + 2e^- \square 2OH^- + H_2 \square$$

As this OH- ion permeates the membrane 115 and passes through, a part

thereof donates an electron e- to the cathode plate 117 to become oxygen gas dissolved in the generated electrolyzed water on the anode side. This causes the electrolyzed water generated on the anode side (i.e., inside the electrolysis chamber 113) to become oxidizing potential water having a higher oxidation/reduction potential (ORP) than

5   electrolyzed water generated using conventional membrane electrolysis technology.

In addition, since the remainder of the OH- ion passed through the membrane 115 reacts with the H+ ion in the electrolysis chamber 113 and reverts to water, the pH of the oxidizing potential water generated with the electrolysis chamber 113 changes slightly towards neutrality. In other words, oxidizing potential water having a pH

10   that is not very low yet having a high ORP is obtained. The oxidizing potential water including the hydrogen ion generated in this manner is supplied from the outlet 112.

Incidentally, continuous water flow electrolysis processing using the reducing potential water generation apparatus 11 shown in FIG. 5 was carried out under electrolysis conditions where the cathodes (-) of the direct-current power source 12 are

15   connected to the two electrode plates 117 and 117 arranged inside the electrolysis chamber 113, the anodes (+) of the direct-current power source 12 are connected to the electrode plates 116 and 116 arranged outside the electrolysis chamber 113 (electrode plate effective surface area is 1 dm^2), and a 5 A constant current is passed through Fujisawa City tap water having a pH of 7.9, ORP of 473 mV and flowing at a rate of 1

20   liter per minute (a preferable flowing rate in the present reducing potential water generation apparatus 11 is 1 through 3 liters per minute, preferably, 1 through 1.8 liters per minute, more preferably, 1.3 through 1.8 liters per minute.) Here, a cation-exchange film made by DuPont(tm), the Nafion(R) Membrane, was used as the membrane 115, the distance between the electrode plates 116 and 117 was 1.2 mm,

25   and the distance between the electrode plates 117 and 117 inside the electrolysis chamber 113 was 1.4 mm.

As a result, a reducing potential water with a pH of 9.03 and ORP of −720 mV was obtained immediately following electrolysis processing. This reducing potential water was left to stand and the pH and ORP were measured after 5 minutes, 10

30   minutes, and 30 minutes. The following results were obtained: after 5 minutes, pH = 8.14 and ORP = -706 mV; after 10 minutes, pH = 8.11 and ORP = -710 mV; and after 30 minutes, pH = 8.02 and ORP = -707 mV. In other words, at the time point immediately following electrolysis processing, the pH of the processing water was higher than 9 but then the pH dropped shortly thereafter, and stabilized near pH 8.

This may emanate from the fact that the H+ ion generated near the water screen between the membrane 115 and the anode plate 116 passes through the membrane 115, moves to the electrolysis chamber 113, and then undergoes a neutralization reaction with the OH- ion in this electrolysis chamber 113 to revert to the previous

5   water.   This neutralization reaction progresses with time to reach chemical equilibrium in concentration, even when the reducing potential water is left standing following electrolysis processing.

Reduction activity/ radical scavenging evaluation testing for precious metal colloid catalyst-containing hydrogen-dissolved water

10   In the following, various evaluation tests of reduction activity and radical scavenging activity as expressed through the chemical activation of inert molecular hydrogen in hydrogen-dissolved water when a precious metal colloid catalyst (platinum (Pt) colloid/ palladium (Pd) colloid) is included in the hydrogen-dissolved water of the present invention are shown through both working examples and reference examples,

15   respectively.

In the two forms of evaluation testing mentioned above, the reduction activity evaluation testing uses methylene blue (tetramethylthionine chloride: $C_{16}H_{18}ClN3S\cdot3(H_2O)$) as the antioxidation subject; on the other hand, in the radical scavenging activity evaluation testing, a radical that is relatively stable in aqueous solution, the

20   DPPH radical (1,1-diphenyl-2-picrylhydrazyl) is used as the antioxidation subject.

Here, to describe the principle behind reduction activity evaluation for the case where methylene blue, which is categorized as an oxidation/reduction pigment, is used as the antioxidation subject, the oxidized methylene blue solution (local maximum absorption wavelength of approximately 665 nm; hereafter methylene blue is also

25   referred to as 'MB') takes on a blue color, however, when this is subjected to reduction and becomes reduced methylene blue (leucomethylene blue), the color changes from the blue color to being colorless.   The degree to which this blue color disappears estimates the reduction activity or in other words, the reducing power.   It should be noted that while the reduced methylene blue produces a white deposit due to low solubility, as it

30   becomes oxidized again, it becomes oxidized methylene blue and the blue color returns. That is, the color change reaction of the methylene blue solution is reversible.

Meanwhile, to describe the principle behind radical scavenging activity evaluation for the case where a DPPH radical is used as the antioxidation subject, the DPPH radical solution (local maximum absorption wavelength of approximately 520

nm; hereafter may be referred to as 'DPPH') takes on a deep red color, and as this DPPH is reduced and no longer a radical, this deep red color fades. The degree to which the color fades estimates the radical scavenging activity or in other words, the antioxidation power. It should be noted that the color change reaction of the DPPH radical solution is nonreversible.

5

The description of these evaluation tests will be made in the following order:

(1)   Reduction activity evaluation of Pt colloid catalyst-containing electrolyzed water using methylene blue color change

(2)   Reduction activity evaluation of Pt colloid/Pd colloid catalyst-containing hydrogen-dissolved water (degasification treatment + hydrogen gas inclusion treatment) using methylene blue color change

10

(3)   Reduction activity evaluation of Pt colloid catalyst-added electrolyzed water (pre-electrolysis processing addition/post-electrolysis processing addition) using methylene blue color change

(4)   Antioxidation activity evaluation of Pt colloid catalyst-containing electrolyzed water using color change of the DPPH radical

15

(5)   Antioxidation activity evaluation of catalyst-containing hydrogen-dissolved water (degasification treatment + hydrogen gas inclusion treatment) using color change of the DPPH radical.

20   (1)   Reduction activity evaluation of Pt colloid catalyst-containing electrolyzed water using methylene blue color change

(1-A): Reducing power evaluation testing procedures

Standard buffer solutions 6.86 (phosphate solution) and 9.18 (borate solution) manufactured by Wako Pure Chemical Industries, Ltd. are respectively diluted to one-tenth strength in purified water to prepare pH buffer solutions. In the following, these two types of dilution water are respectively referred to as 'base water 6.86' and 'base water 9.18'. In addition, a solution having 0.6 g of a Tanaka Kikinzoku-manufactured platinum colloid (particle size distribution is 2 through 4 nm, including polyvinylpyrrolidone as a dispersion agent) 4% solution dissolved in 500 mL of

25

30   distilled water manufactured by Wako Pure Chemical Industries, Ltd. is referred to as 'Pt standard solution'. It should be noted that the platinum component concentration C(Pt) in the Pt standard solution becomes a 48 mg/L concentration using the formula C(Pt) = 0.6 g x 0.04 /500 mL. Then using either base water 6.86 or base water 9.18 of the two species described above with the Pt standard solution, a total of eight species of

sample solution, four species each, are prepared. These are described below:

i. Base water (6.86)

ii. Pt colloid-containing solution, where 6 mL of Pt standard solution is added to 1494 mL of base water (6.86)

5   iii. A solution where base water (6.86) has been subjected to electrolysis processing

iv. A solution where 6 mL of Pt standard solution is added to 1494 mL of base water (6.86) to make a Pt colloid-containing solution, and this solution is subjected to electrolysis processing

v. Base water (9.18)

10 vi. Pt colloid-containing solution, where 6 mL of Pt standard solution is added to 1494 mL of base water (9.18)

vii. A solution where base water (9.18) has been subjected to electrolysis processing

viii. A solution where 6 mL of Pt standard solution is added to 1494 mL of base water (9.18) to make a Pt colloid-containing solution, and this solution is subjected to

15 electrolysis processing

It should be noted that the pH, ORP (mV), temperature T (°C), and Pt colloid concentration for each sample solution of the total 8 described above in i through viii are collectively shown in the following Table 2.

[Table 2]

20 ★

In order to examine the respective reduction activity of each sample solution of the total 8 described above in i through viii, 10 mL of methylene blue (1 g/L concentration) is added to 350 mL of each solution to prepare a methylene blue mole concentration of 74.4 μM, and the methylene blue light absorbance (A589: the light

25 absorbance at wavelength 589 nm) of each sample solution is measured using a spectrophotometer.

(1-B): Disclosure of reference examples and working examples

(Reference Example 1)

The methylene blue light absorbance (A589) of a solution where methylene

30 blue is added to the catalyst-free solution (base water 6.86) of sample i is given as Reference Example 1, and the result thereof is shown in FIG. 6.

(Reference Example 2)

The methylene blue light absorbance (A589) of a solution where methylene blue is added to the catalyst-containing solution (base water 6.86 + Pt standard

solution) of sample ii is given as Reference Example 2, and the result thereof is shown in FIG. 6.

(Reference Example 3)

5   The methylene blue light absorbance (A589) of a solution where methylene blue is added to the catalyst-free electrolyzed water (base water 6.86 + electrolysis processing) of sample iii is given as Reference Example 3, and the result thereof is shown in FIG. 6.

(Working Example 1)

The methylene blue light absorbance (A589) of a solution where methylene

10   blue is added to the catalyst-containing electrolyzed water (base water 6.86 + electrolysis processing + Pt standard solution) of sample iv is given as Working Example 1, and the result thereof is shown in FIG. 6 for comparison with Reference Examples 1 through 3.

(Reference Example 4)

15   The methylene blue light absorbance (A589) of a solution where methylene blue is added to the catalyst-free solution (base water 9.18) of sample v is given as Reference Example 4, and the result thereof is shown in FIG. 7.

(Reference Example 5)

The methylene blue light absorbance (A589) of a solution where methylene

20   blue is added to the catalyst-containing solution (base water 9.18 + Pt standard solution) of sample vi is given as Reference Example 5, and the result thereof is shown in FIG. 7.

(Reference Example 6)

The methylene blue light absorbance (A589) of a solution where methylene

25   blue is added to the catalyst-free electrolyzed water (base water 9.18 + electrolysis processing) of sample vii is given as Reference Example 6, and the result thereof is shown in FIG. 7.

(Working Example 2)

The methylene blue light absorbance (A589) of a solution where methylene

30   blue is added to the catalyst-containing electrolyzed water (base water 9.18 + electrolysis processing + Pt standard solution) of sample viii is given as Working Example 2, and the result thereof is shown in FIG. 7 for comparison with Reference Examples 4 through 6.

(1-C): Examination of working examples

Examining the results of Working Examples 1 and 2 in comparison with those of Reference Examples 1 through 6, it may be said that the catalyst-containing electrolyzed waters of Working Examples 1 and 2 has the specific methylene blue reduced irrespective of the difference in pH thereof, yet only the catalyst-containing electrolyzed water exhibits significant reduction activity. It should be noted that when it was visually checked whether or not there had been a change in the blue color of the methylene blue solution, only the catalyst-containing electrolyzed waters of Working Examples 1 and 2 were colorless and clear, allowing visual confirmation that the blue color of the methylene blue had disappeared. However, visual confirmation that the blue color of the methylene blue had disappeared could not be accomplished with Reference Examples 1 through 6. In addition, a large amount of white-colored deposit (reduced methylene blue) was visually confirmed for the catalyst-containing hydrogen-dissolved waters of Working Examples 1 and 2.

(2)   Reduction activity evaluation of Pt colloid/Pd colloid catalyst-containing hydrogen-dissolved water (degasification treatment + hydrogen gas inclusion treatment) using methylene blue color change

(2-A): Reducing power evaluation testing procedures

Solutions of Tris-HCl with a concentration of 50mM are prepared by respectively diluting a special order 1M Tris-HCl (pH 7.4) and a special order 1M Tris-HCl (pH 9.0) manufactured by Nippon Gene Co., Ltd. and sold by Wako Pure Chemical Industries, Ltd. to one-twentieth strength with distilled water manufactured by Wako Pure Chemical Industries, Ltd. In the following, these two types of dilution water are respectively referred to as 'base water 7.4' and 'base water 9.0'. In addition, a solution having 0.6 g of a Tanaka Kikinzoku-manufactured palladium colloid (particle size distribution is 2 to 4 nm, including polyvinylpyrrolidone as a dispersion agent) 4% solution dissolved in 500 mL of distilled water manufactured by Wako Pure Chemical Industries, Ltd. is referred to as 'Pd standard solution'. It should be noted that the palladium component concentration C(Pd) in the Pd standard solution becomes a 48 mg/L concentration using, from the same formula as the Pt colloid, C(Pd) = 0.6 g x 0.04 /500 mL.

Next, collecting 84 mL of base water 7.4 and base water 9.0, respectively, 4 mL of MB solution in 1 g/L concentration is added to each to prepare base water 7.4 and base water 9.0 that respectively contain a 121.7 μM concentration of methylene blue (MB). 50 mL of each of these MB-containing base waters 7.4 and 9.0 are further

collected into individual degasification bottles and subjected three times to a process that includes 10 minute degasification with a vacuum pump followed by 10 minute hydrogen gas inclusion. This process aims to remove gaseous components other than hydrogen from the hydrogen-dissolved water.

5   3 mL of the respective hydrogen gas-included, MB-containing base water 7.4 and base water 9.0 obtained in this manner is collected and poured into respective sealed, hydrogen gas-replaced, quartz cells. Measurements are then taken of the change in methylene blue light absorbance ($\square$A572: change in light absorbance at wavelength 572 nm) that occurs when the Pt reference solution, Pd standard solution,

10   or mixed solution of Pt standard solution and Pd standard solution with a mole ratio of approximately 1 is respectively added to the quartz cells.

(2-B): Disclosure of working examples

(Working Example 3)

        The change in MB light absorbance ($\square$A572) in a solution where an amount of

15   Pt standard solution sufficient to give a Pt colloid concentration of 190 μg/L has been added to MB-containing hydrogen-dissolved water (MB-containing base water 7.4 + degasification treatment + hydrogen gas inclusion treatment) is given as Working Example 3, and the result thereof is shown in both FIG. 8 and FIG. 9.

(Working Example 4)

20   The change in MB light absorbance ($\square$A572) in a solution where an amount of Pt standard solution sufficient to give a Pt colloid concentration of 190 μg/L has been added to MB-containing hydrogen-dissolved water (MB-containing base water 9.0 + degasification treatment + hydrogen gas inclusion treatment) is given as Working Example 4, and the result thereof is shown in FIG. 8 for comparison with Working

25   Example 3. It should be noted that the difference between the sample waters of Working Example 3 and Working Example 4 is the pH.

(Working Example 5)

        The change in MB light absorbance ($\square$A572) in a solution where an amount of Pt standard solution sufficient to give a Pt colloid concentration of 95 μg/L has been

30   added to MB-containing hydrogen-dissolved water (MB-containing base water 7.4 + degasification treatment + hydrogen gas inclusion treatment) is given as Working Example 5, and the result thereof is shown in FIG. 9 for comparison with Working Example 3. It should be noted that the difference between the sample waters of Working Example 3 and Working Example 5 is the Pt colloid concentration.

(Working Example 6)

The change in MB light absorbance ($\Box$A572) in a solution where an amount of Pd standard solution sufficient to give a palladium colloid concentration of 444 µg/L has been added to MB-containing hydrogen-dissolved water (MB-containing base water 7.4

5 + degasification treatment + hydrogen gas inclusion treatment) is given as Working Example 6, and the result thereof is shown in both FIG. 10 and FIG. 11.

(Working Example 7)

The change in MB light absorbance ($\Box$A572) in a solution where an amount of Pd standard solution sufficient to give a palladium colloid concentration of 444 µg/L has been

10 added to MB-containing hydrogen-dissolved water (MB-containing base water 9.0 + degasification treatment + hydrogen gas inclusion treatment) is given as Working Example 7, and the result thereof is shown in FIG. 10 for comparison with Working Example 6. It should be noted that the difference between the sample waters of Working Example 6 and Working Example 7 is the pH.

15 (Working Example 8)

The change in MB light absorbance ($\Box$A572) in a solution where an amount of Pd standard solution sufficient to give a palladium colloid concentration of 111 µg/L has been added to MB-containing hydrogen-dissolved water (MB-containing base water 7.4 + degasification treatment + hydrogen gas inclusion treatment) is given as Working

20 Example 8, and the result thereof is shown in FIG. 11 for comparison with Working Example 6. It should be noted that the difference between the sample waters of Working Example 6 and Working Example 8 is the palladium colloid concentration.

(Working Example 9)

The change in MB light absorbance ($\Box$A572) in a solution where an amount of a

25 mixed solution of Pt standard solution and Pd standard solution with a mole ratio of approximately 1 sufficient to give a precious metal mixed (Pt + Pd) colloid concentration of 160 µg/L has been added to MB-containing hydrogen-dissolved water (MB-containing base water 7.4 + degasification treatment + hydrogen gas inclusion treatment) is given as Working Example 9, and the result thereof is shown in both FIG. 12 and FIG. 13.

30 (Working Example 10)

The change in MB light absorbance ($\Box$A572) in a solution where an amount of mixed solution, similar to Working Example 9, sufficient to give a precious metal mixed (Pt + Pd) colloid concentration of 160 µg/L has been added to MB-containing hydrogen-dissolved water (MB-containing base water 9.0 + degasification treatment +

hydrogen gas inclusion treatment) is given as Working Example 10, and the result thereof is shown in FIG. 12 for comparison with Working Example 9. It should be noted that the difference between the sample waters of Working Example 9 and Working Example 10 is the pH.

5 (Working Example 11)

The change in MB light absorbance ($\Box$A572) in a solution where an amount of mixed solution, similar to Working Example 9, sufficient to give a precious metal mixed (Pt + Pd) colloid concentration of 80 µg/L has been added to MB-containing hydrogen-dissolved water (MB-containing base water 7.4 + degasification treatment +

10 hydrogen gas inclusion treatment) is given as Working Example 11, and the result thereof is shown in FIG. 13 for comparison with Working Example 9. It should be noted that the difference between the sample waters of Working Example 9 and Working Example 11 is the precious metal (Pt + Pd) colloid concentration.

(2-C): Examination of working examples

15 FIG. 8, which compares Working Examples 3 and 4, shows the MB reduction activity of Pt colloid-added hydrogen-dissolved water occurring at pH 7.4 and pH 9.0. According to this diagram, both examples show high levels of MB reduction activity without seeing a substantial difference in MB reduction activity due to difference in pH.

FIG. 9, which compares Working Examples 3 and 5, shows the MB reduction

20 activity of Pt colloid-added hydrogen-dissolved water occurring at Pt colloid concentrations of 95 µg/L and 190 µg/L. According to this diagram, the higher Pt colloid concentration also has higher MB reduction activity. From this, an increase in Pt colloid concentration may be effective towards increasing MB reduction activity.

FIG. 10, which compares Working Examples 6 and 7, shows the MB reduction

25 activity of Pd colloid-added hydrogen-dissolved water occurring at pH 7.4 and pH 9.0. According to this diagram, both examples show high levels of MB reduction activity without seeing a substantial difference in MB reduction activity due to difference in pH.

FIG. 11, which compares Working Examples 6 and 8, shows the MB reduction activity of Pd colloid-added hydrogen-dissolved water occurring at Pd colloid

30 concentrations of 111 µg/L and 444 µg/L. According to this diagram, the higher Pd colloid concentration also has higher MB reduction activity. From this, an increase in Pd colloid concentration may be effective towards increasing MB reduction activity.

FIG. 12, which compares Working Examples 9 and 10, shows the MB reduction activity of precious metal mixed (Pt + Pd) colloid-added hydrogen-dissolved water

occurring at pH 7.4 and pH 9.0. According to this diagram, both examples show high levels of MB reduction activity without seeing a substantial difference in MB reduction activity due to difference in pH.

FIG. 13, which compares Working Examples 9 and 11, shows the MB reduction activity of precious metal mixed (Pt + Pd) colloid-added hydrogen-dissolved water occurring at precious metal mixed (Pt + Pd) colloid concentrations of 80 μg/L and 160 μg/L. According to this diagram, the higher precious metal mixed (Pt + Pd) colloid concentration also has higher MB reduction activity. From this, an increase in precious metal mixed (Pt + Pd) colloid concentration may be effective towards increasing MB reduction activity.

In addition, comparing FIG. 8 (Working Examples 3 and 4: MB reduction activity of Pt colloid-added hydrogen-dissolved water) and FIG. 10 (Working Examples 6 and 7: MB reduction activity of Pd colloid-added hydrogen-dissolved water), it may be understood that although Working Examples 3 and 4 have lower concentrations, these show substantially the same MB reduction activity as Working Examples 6 and 7. Moreover, comparing the mole concentrations (μM) of both, since the Pt colloid is 0.98 μM and the Pd colloid 4.17 μM, the Pt colloid uses a lower mole concentration. This means that regarding MB reduction activity expected for the precious metal catalyst according to the present invention, it may be said that the Pt colloid is superior to the Pd colloid in terms of catalytic activity because substantially the same MB reduction activity can be obtained with a smaller dosage.

Meanwhile, comparing FIG. 8 (Working Examples 3 and 4: MB reduction activity of Pt colloid-added hydrogen-dissolved water) and FIG. 12 (Working Examples 9 and 10: MB reduction activity of precious metal mixed (Pt + Pd) colloid-added hydrogen-dissolved water), it may be understood that both show superior MB reduction activity. Even comparing the mole concentrations (μM) of both, since the Pt colloid is 0.98 μM and the precious metal mixed (Pt + Pd) colloid 1.07 μM, both are substantially the same. Therefore, regarding MB reduction activity expected for the precious metal catalyst according to the present invention, the Pt colloid and the precious metal mixed (Pt + Pd) colloid are substantially the same in terms of catalytic activity.

(3)   Reduction activity evaluation of Pt colloid catalyst-containing electrolyzed water (pre-electrolysis processing addition/post-electrolysis processing addition) using methylene blue color change

(3-A): Reducing power evaluation testing procedures

2000 mL of base water 6.86 similar to that prepared in (1-A) described above is prepared, and 4 mL of Pt standard solution from this is added to 1000 mL to prepare approximately 1 liter of Pt colloid-containing base water 6.86. For the time being, the Pt colloid is not added to the remaining 1000 mL. In this manner, approximately 1 liter of Pt colloid-free base water 6.86 and approximately 1 liter of Pt colloid-containing base water 6.86 are prepared.

Next, both of the samples are subjected to electrolysis processing separately. 2.86 mL of the respective obtained electrolyzed waters (hydrogen-dissolved water) is collected and poured into respective sealed, hydrogen gas-replaced quartz cells.

Moreover, only 0.14 mL of the 1 g/L concentration MB solution that has been degasified and hydrogen gas included beforehand is added to the Pt colloid-free cell. At this point, both cells are set in the spectrophotometer and placed on stand-by.

Next, 12 μL in a 48 mg/L concentration of Pt colloid solution is added to the Pt colloid-free cell, and into the Pt colloid-containing cell, 0.14 mL of 1 g/L concentration MB solution that has already been through degasification treatment and hydrogen gas inclusion treatment is added, and measurement of both cell solutions is begun. It should be noted that the Pt colloid concentrations added to each cell are prepared so that each respectively becomes approximately 182 μg/L.

(3-B): Disclosure of working examples

(Working Example 12)

The minimum value of MB light absorbance (A572: the light absorbance at wavelength 572 nm) of the pre-electrolysis catalyst-added electrolyzed water (MB-containing base water 6.86 + Pt colloid pre-electrolysis addition) that occurs within 30 minutes from the start of measurement is given as Working Example 12, and the result thereof is shown in FIG. 14.

(Working Example 13)

The minimum value of MB light absorbance (A572) of the post-electrolysis catalyst-added electrolyzed water (MB-containing base water 6.86 + Pt colloid post-electrolysis addition) that occurs within 30 minutes from the start of measurement is given as Working Example 13, and the result thereof is shown in FIG. 14 for comparison with Working Example 12.

(3-C): Examination of working examples

FIG. 14, which compares Working Examples 12 and 13, shows the MB reduction activity of electrolyzed water when the period of adding the Pt colloid is

different (before vs. after electrolysis processing). According to this diagram, it may be understood that adding the Pt colloid before electrolysis processing allows higher MB reduction activity to be obtained. The reason for this is still being studied, however it is speculated that this stems from the activated hydrogen at the root of the MB

5 reduction activity making the oxidizing power of the oxidant such as oxygen in the electrolyzed water ineffective. This is the reason derived from the fact that when the dissolved oxygen concentration of the electrolyzed water on which electrolysis processing had been implemented using Pt colloid-containing activated carbon processing water as the raw water was measured immediately after electrolysis

10 processing thereof, the concentration of dissolved oxygen in this electrolyzed water was found to be substantially zero. Should this be the case, not only in this exemplary electrolysis processing, but also in hydrogen inclusion treatment or hydrogen gas bubbling processing, addition of the catalyst (Pt colloid) before processing may be preferable from the standpoint that higher levels of MB reduction activity are obtained

15 (because of the oxidizing power of the oxidant such as oxygen being made ineffective). Moreover, even in the case of obtaining dissolved hydrogen water by employing processing where, for instance, a reducing agent is added to the raw water, addition of the Pt colloid to the raw water beforehand may be preferable from the standpoint that higher levels of MB reduction activity similar to that described above may be obtained.

20 It should be noted that the catalyst is not limited to the Pt colloid. Addition of a catalyst such as Pd colloid, or mixed colloid of Pt colloid and Pd colloid before processing is similarly preferable from the standpoint of obtaining higher levels of MB reduction activity (catalytic activity). This emanates from the fact that hydrogen may be efficiently occluded into a precious metal colloid catalyst during electrolysis processing

25 when the precious metal colloid catalyst is added prior to that electrolysis processing, and the hydrogen stored in the precious metal colloid catalyst allows even higher levels of MB reduction activity (catalytic activity).

(4)   Antioxidation activity evaluation of Pt colloid catalyst-containing electrolyzed water using color change of the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical

30   The free radical DPPH is inactivated by becoming a non-radical through a reaction with an antioxidant, reducing light absorbance in the vicinity of wavelength 520 nm. Measurement of this reduction allows measurement of radical scavenging activity of the antioxidant.

(4-A): Antioxidation activity evaluation testing procedures

In order to examine the respective antioxidation activity of each sample solution of the total 8 samples i through viii shown in table 2, similar to that prepared in (1-A) above, 4 mL of DPPH (0.16 g/L concentration) is added to 16 mL of each solution to prepare a DPPH mole concentration of 81.15 (μM), and the change in DPPH light

5 absorbance (A540: the light absorbance at wavelength 540 nm) of each solution three minutes after adding the DPPH is measured using a spectrophotometer.

(4-B): Disclosure of reference examples and working examples

(Reference Example 7)

The difference in DPPH light absorbance (□A540) of a solution where DPPH is

10 added to the catalyst-free solution (base water 6.86) of sample i is given as Reference Example 7, and the result thereof is shown in FIG. 15. It should be noted that the change in DPPH light absorbance (□A540) in the same drawing shows the difference (□A540) between the light absorbance of this sample i (blank) and the light absorbance of samples i through iv. Accordingly, the change in DPPH light absorbance (□A540) for

15 Reference Example 7 is zero.

(Reference Example 8)

The change in DPPH light absorbance (□A540) of a solution where DPPH is added to the catalyst-containing solution (base water 6.86 + Pt standard solution) of sample ii is given as Reference Example 8, and the result thereof is shown in FIG. 15.

20 (Reference Example 9)

The change in DPPH light absorbance (□A540) of a solution where DPPH is added to the catalyst-free solution (base water 6.86 + electrolysis processing) of sample iii is given as Reference Example 9, and the result thereof is shown in FIG. 15.

(Working Example 14)

25 The change in DPPH light absorbance (□A540) of a solution where DPPH is added to the catalyst-added electrolyzed water (base water 6.86 + electrolysis processing + Pt standard solution) of sample iv is given as Working Example 14, and the result thereof is shown in FIG. 15 for comparison with Reference Examples 7 through 9.

(Reference Example 10)

30 The change in DPPH light absorbance (□A540) of a solution where DPPH is added to the catalyst-free solution (base water 9.18) of sample v is given as Reference Example 10, and the result thereof is shown in FIG. 16. It should be noted that the change in DPPH light absorbance (□A540) in the same drawing shows the difference (□A540) between the light absorbance of this sample v (blank) and the light absorbance

of samples v through viii. Accordingly, the change in DPPH light absorbance ($\square$A540) for Reference Example 10 is zero.

(Reference Example 11)

The change in DPPH light absorbance ($\square$A540) of a solution where DPPH is added to the catalyst-containing solution (base water 9.18 + Pt standard solution) of sample vi is given as Reference Example 11, and the result thereof is shown in FIG. 16.

(Reference Example 12)

The change in DPPH light absorbance ($\square$A540) of a solution where DPPH is added to the catalyst-free electrolyzed water (base water 9.18 + electrolysis processing) of sample vii is given as Reference Example 12, and the result thereof is shown in FIG. 16.

(Working Example 15)

The change in DPPH light absorbance ($\square$A540) of a solution where DPPH is added to the catalyst-containing electrolyzed water (base water 9.18 + electrolysis processing + Pt standard solution) of sample viii is given as Working Example 15, and the result thereof is shown in FIG. 16 for comparison with Reference Examples 10 through 12.

(4-C): Examination of Working Examples

Examining the results of Working Examples 14 and 15 in comparison with those of Reference Examples 7 through 12, it may be said that the catalyst-containing electrolyzed waters of Working Examples 14 and 15 have the specific DPPH radical scavenged with both base waters 6.86 and 9.18, and show significant antioxidation activity and radical scavenging activity. However, the Pt colloid catalyst was added before electrolysis processing. It should be noted that, as shown in FIG. 15, DPPH radical scavenging activity is found in Reference Example 9 even though catalyst-free electrolyzed water is used. This indicates that with high-concentration hydrogen-dissolved water such as electrolyzed water disclosed in this specification, when an oxidation substance to be reduced is a radical having a strong oxidizing power, an expression of radical scavenging activity is expected to occur through forcible hydrogen degasification reaction from molecular hydrogen with that radical, even without the assistance of a catalyst.

(5) Antioxidation activity evaluation of catalyst-containing hydrogen-dissolved water (degasification treatment + hydrogen gas inclusion treatment) using color change of the DPPH radical

(5-A): Antioxidation activity evaluation testing procedures

'Base water 7.4' and 'base water 9.0' are prepared as with that prepared in (2-A) above. Next, 406 μM of DPPH solution and 50 mL each of base water 7.4 and base water 9.0 are collected and subjected three times to a process that includes 10 minute

5   degasification with a vacuum pump followed by 10 minutes of hydrogen gas inclusion. This process aims to remove gaseous components other than hydrogen from the hydrogen-dissolved water.

0.3 mL of the hydrogen gas-included DPPH solution obtained in this manner, and 2.7 mL each of base water 7.4 and base water 9.0 are collected and poured into

10 respective sealed, hydrogen gas-replaced, quartz cells. Measurements of the change in DPPH light absorbance ($\Box$A540: change in light absorbance at wavelength 540 nm) for both that to which the Pt standard solution has been added and that to which it has not are then taken over 30 minutes respectively using a spectrophotometer.

(5-B): Disclosure of reference examples and working examples

15 (Reference Example 13)

The change in DPPH light absorbance ($\Box$A540) of a solution where Pt standard solution has not been added to the hydrogen-dissolved water (base water 7.4 + degasification treatment + hydrogen gas inclusion treatment) is given as Reference Example 13, and the result thereof is shown in FIG. 17.

20 (Working Example 16)

The change in DPPH light absorbance ($\Box$A540) in a solution where an amount of Pt standard solution sufficient to give a Pt colloid concentration of 190 μg/L has been added to hydrogen-dissolved water (base water 7.4 + degasification treatment + hydrogen gas inclusion treatment) is given as Working Example 16, and the result

25 thereof is shown in FIG. 17 for comparison with Reference Example 13. It should be noted that the difference between Reference Example 13 and Working Example 16 is whether or not the Pt colloid has been added.

(Reference Example 14)

The change in DPPH light absorbance ($\Box$A540) of a solution where Pt standard

30 solution has not been added to the hydrogen-dissolved water (base water 9.0 + degasification treatment + hydrogen gas inclusion treatment) is given as Reference Example 14, and the result thereof is shown in FIG. 18.

(Working Example 17)

The change in DPPH light absorbance ($\Box$A540) in a solution where an amount

of Pt standard solution sufficient to give a Pt colloid concentration of 190 µg/L has been added to hydrogen-dissolved water (base water 9.0 + degasification treatment + hydrogen gas inclusion treatment) is given as Working Example 17, and the result thereof is shown in FIG. 18 for comparison with Reference Example 14. It should be noted that the difference between Reference Example 14 and Working Example 17 is whether or not the Pt colloid has been added.

(5-C): Examination of working examples

FIG. 17, which compares Reference Example 13 and Working Example 16, shows the DPPH radical scavenging activity in pH 7.4 hydrogen-dissolved water where the difference is whether or not the Pt colloid is added. FIG. 18, which compares Reference Example 14 and Working Example 17, shows the DPPH radical scavenging activity in pH 9.0 hydrogen-dissolved water where the difference is whether or not the Pt colloid is added. According to these diagrams, with the Pt colloid-free Reference Examples 13 and 14, the change in light absorbance observed may correspond to only natural fading during the duration of measurement (30 minutes). Meanwhile, with the Pt colloid-containing Working Examples 16 and 17, the expression of DPPH radical scavenging that clearly surpasses natural fading is observed. It should be noted that there was no substantial difference observed in levels of DPPH radical scavenging due to difference in pH.

Reduction activity evaluation testing of enzyme hydrogenase catalyst-containing hydrogen-dissolved water

Next, evaluation of reduction activity as expressed through the chemical activation of inert molecular hydrogen in hydrogen-dissolved water when an enzyme hydrogenase catalyst is included in the hydrogen-dissolved water of the present invention is shown through both working examples and reference examples, respectively. In this reduction activity evaluation test, the oxidation/reduction pigment methylene blue is used as the antioxidation subject as with the reduction activity testing for precious metal colloid catalyst-containing hydrogen-dissolved water. Since the reduction activity evaluation principle in this case is similar to that described for the precious metal colloid catalyst above, repetitive description thereof is omitted.

(6)   Reduction activity evaluation of enzyme hydrogenase catalyst-containing hydrogen-dissolved water (degasification treatment + hydrogen gas inclusion treatment) using methylene blue color change

(6-A): Reduction activity evaluation testing procedures

In the same manner as that prepared in (2-A) above, 'base water 7.4' and 'base water 9.0' are prepared. Next, collecting 84 mL of each of base water 7.4 and base water 9.0, respectively, 4 mL of MB solution in 1 g/L concentration is added to each to prepare base water 7.4 and base water 9.0 that respectively contain a 121.7 μM

5   concentration of methylene blue (MB). 50 mL of each of these MB-containing base waters 7.4 and 9.0 are further collected and subjected three times to a process that includes 10 minute degasification with a vacuum pump followed by 10 minute hydrogen gas inclusion. This process aims to remove gaseous components other than hydrogen from the hydrogen-dissolved water. Meanwhile, a 125 μM concentration of

10   hydrogenase solution is diluted with distilled water to one-fourth strength. This is then poured into 1 mL microcapsules and the oxygen is removed by infusing these capsules with nitrogen gas (inert gas).

3 mL of the respective hydrogen gas-included, MB-containing base water 7.4 and base water 9.0 obtained in this manner is collected and poured into respective

15   sealed, hydrogen gas-replaced, quartz cells. Measurements are then taken of the change in methylene blue light absorbance (□A572) that occurs when the hydrogenase solution prepared as described above is added to the quartz cells.

(6-B): Disclosure of reference examples and working examples

(Working Example 18)

20   The change in MB light absorbance (□A572) in a solution where 10 μL of the hydrogenase solution prepared as described above has been added to MB-containing hydrogen-dissolved water (MB-containing base water 7.4 + degasification treatment + hydrogen gas inclusion treatment) is given as Working Example 18, and the result thereof is shown in FIG. 19.

25   (Reference Example 15)

The change in MB light absorbance (□A572) in a solution where the hydrogenase solution has not been added to MB-containing hydrogen-dissolved water (MB-containing base water 7.4 + degasification treatment + hydrogen gas inclusion treatment) is given as Reference Example 15, and the result thereof is shown in FIG. 19

30   for comparison with Working Example 18. It should be noted that the difference between the sample waters of Working Example 18 and Reference Example 15 is whether or not the enzyme hydrogenase has been included.

(Working Example 19)

The change in MB light absorbance (□A572) in a solution where 10 μL of the

hydrogenase solution prepared as described above has been added to MB-containing hydrogen-dissolved water (MB-containing base water 9.0 + degasification treatment + hydrogen gas inclusion treatment) is given as Working Example 19, and the result thereof is shown in FIG. 20.

5 (Reference Example 16)

The change in MB light absorbance ($\Box$A572) in a solution where the hydrogenase solution has not been added to MB-containing hydrogen-dissolved water (MB-containing base water 9.0 + degasification treatment + hydrogen gas inclusion treatment) is given as Reference Example 16, and the result thereof is shown in FIG. 20 10 for comparison with Working Example 19. It should be noted that the difference between the sample waters of Working Example 19 and Reference Example 16 is whether or not the enzyme hydrogenase has been included.

(6-C): Examination of working examples

Examining the results of Working Examples 18 and 19 in comparison with 15 those of Reference Examples 15 and 16, it may be said that the catalyst-containing hydrogen-dissolved waters of Working Examples 18 and 19 have the methylene blue specifically reduced irrespective of the difference in pH thereof, yet only the catalyst-containing hydrogen-dissolved water exhibits significant reduction activity. It should be noted that when it was visually checked whether or not there had been a 20 change in the blue color of the methylene blue solution, only the catalyst-containing hydrogen-dissolved waters of Working Examples 18 and 19 were colorless and clear, allowing visual confirmation that the blue color of the methylene blue had disappeared. However, visual confirmation that the blue color of the methylene blue had disappeared could not be accomplished with Reference Examples 15 and 16. In addition, a large 25 amount of white-colored deposit (reduced methylene blue) was visually confirmed for the catalyst-added hydrogen-dissolved waters of Working Examples 18 and 19.

Quantitative analysis of dissolved hydrogen concentration through oxidation/reduction titration of oxidation/reduction pigment

(A) Development of idea

30 It has been proven that hydrogen generated through the negative electrode reaction during electrolysis processing is dissolved in the electrolyzed water (electrolyzed reducing water) that has been subjected to electrolysis processing in the reducing potential water generation apparatus 11 developed by the applicants. Approximately what concentration of hydrogen is dissolved in this electrolyzed water

may be measured in a way with a dissolved hydrogen meter. Here, the expression 'in a way' is used because generally used dissolved hydrogen meters employ a measuring principle whereby electrochemical physical quantities occurring in the electrode reaction are replaced with the concentration of dissolved hydrogen using a table look-up

5   protocol so that the readings tend to vary significantly depending on the external causes such as liquid properties of the test water.

However, as description was made based on the working examples already described above, with the catalyst-free electrolyzed water, even when an oxidation/reduction pigment (an antioxidation subject) such as oxidized methylene blue

10   is added, this pigment does not show the color change specific to the reduction reaction; but on the other hand, with catalyst-containing electrolyzed water, when this pigment is added, the pigment shows the color change specific to the reduction reaction. In other words, the oxidation/reduction reaction of the oxidation/reduction pigment may be visually recognized by observing the change in color of the solution (catalyst-containing

15   electrolyzed water + oxidation/reduction pigment).

Through a process of trial and error as this testing was repeated, the inventors realized that the color change reaction of the oxidation/reduction pigment methylene blue from blue to clear tended to occur more swiftly as the reducing power of the catalyst-containing electrolyzed water increased. More specifically, when comparing

20   the reducing power of the catalyst-containing electrolyzed water and the reducing power consumed to reduce the oxidation/reduction pigment methylene blue that is added, some sort of correlation was noticed between the size of the residual reducing power or the difference between the two reducing powers when the former is larger than the latter, and the speed of the color change reaction of the oxidation/reduction pigment

25   methylene blue.

In keeping with this discovery, as zealous research on the possible industrial utilization of this correlation progressed, the inventors ended up wondering if it was possible to perform quantitative analysis of the explicit antioxidation power (dissolved hydrogen concentration) of the catalyst-containing electrolyzed water through the

30   oxidation/reduction reaction of the oxidation/reduction pigment methylene blue.

It has been noticed that a dissolved hydrogen concentration quantitative analysis method can be provided as a means of realizing the above-mentioned idea comprising the following: dripping a solution of the oxidation/reduction pigment into a predetermined amount of test water, which includes a precious metal colloid catalyst

during titration under the condition of being isolated from the outside environment; calculating the amount of dissolved hydrogen in test water from the dripped amount of oxidation/reduction pigment up to the end point of the pigment color change through reduction reaction of that oxidation/reduction pigment employing the precious metal colloid catalyst; and performing quantitative analysis of the dissolved hydrogen concentration in test water based on the predetermined amount of test water and the dripped amount of pigment. In addition, it also has been noticed that as an apparatus for performing that analysis, a dissolved hydrogen concentration quantitative analysis apparatus or a gas-impermeable tester isolated from the outside environment is suitably used comprising: a cylinder-shaped tube having a closed and an open end and a pusher capable of being inserted into the cylinder-shaped tube from the open end in a piston-like manner where a stirrer is movable; wherein a solution injection part is deployed on any one of the closed end, the inside wall, or the pusher of this cylinder-shaped tube so as to inject a solution into the test water holding compartment demarcated by the closed end, the inside wall and the pusher of this cylinder-shaped tube under the condition of being isolated from the outside environment. It should be noted that as a modified example of the quantitative analysis method, a dissolved hydrogen concentration quantitative analysis method may be used for performing quantitative analysis of the dissolved hydrogen concentration in test water based on the color change speed of the oxidation/reduction pigment through reduction reaction of that pigment with the assistance of the precious metal colloid catalyst when dripping only a predetermined amount of solution with a predetermined concentration of the oxidation/reduction pigment into a predetermined amount of test water, which includes the precious metal colloid catalyst, instead of the quantitative analysis method based on the total dripped amount until the end point of the oxidation/reduction pigment color change through reduction reaction of that pigment.

(B) Testing objectives

When a solution with a predetermined concentration of oxidation/reduction pigment methylene blue is dripped into the hydrogen-dissolved water that includes catalyst-containing electrolyzed water, the fact that the total dripped amount of methylene blue added until this post-drip solution no longer causes the reducing color reaction to be displayed (hereafter, also referred to as 'end point') becomes a measure of the quantitative analysis of the dissolved hydrogen concentration (explicit antioxidation power) is verified through the following tests.

(C) Outline of effective dissolved hydrogen concentration quantitative analysis method

In order to quantitatively analyze the effective amount of reducing power (antioxidation power) expressed through the chemical activation of inert molecular hydrogen in the hydrogen-dissolved water, or in other words, the effective dissolved
5   hydrogen concentration DH (mg/L) when a catalyst is included in the hydrogen-dissolved water according to the present invention, methylene blue oxidation/reduction titration was carried out on the catalyst (Pt colloid) -added hydrogen-dissolved water using Pt colloid as the catalyst and methylene blue as the oxidation/reduction pigment.

10   (D) Testing procedures

The basic testing procedures include preparing a number of sample waters (already having respective features such as dissolved hydrogen concentration measured), adding the catalyst (Pt colloid) to these samples, and delivering drops of the methylene blue. Comparative evaluation is then made of whether or not there exists
15   correlation between the effective amount of dissolved hydrogen concentration found from each total amount of methylene blue added and the actual reading of the dissolved hydrogen meter.

If there is a correlation between the two, the legitimacy of the dissolved hydrogen concentration quantitative analysis through methylene blue redox titration,
20   and the fact that the key material expressing the explicit antioxidant function is dissolved hydrogen can be objectively validated.

In keeping with such basic thinking, to begin with, a one-fortieth strength Pt standard solution is prepared by diluting the Pt standard solution described earlier to a concentration of one-fortieth strength. It should be noted that the platinum
25   component concentration C(Pt) in this one-fortieth strength Pt standard solution becomes a 192 mg/L concentration using the formula C(Pt) = 24 g x 0.04 /500 mL.

Next, a 1 g/L concentration (mole concentration by volume: 2677.4 μM) of methylene blue solution and a 10 g/L concentration (mole concentration by volume: 26773.8 μM) of methylene blue solution are prepared. Here, two types of different
30   concentrations of methylene blue solution are prepared because changing the concentration of the methylene blue solution to be added in response to the hydrogen concentration which would be dissolved in the water to be tested is expected to result in allowing the added amount of the solution to be reduced and improve test accuracy. Nevertheless, the Pt concentration in the Pt standard solution and the MB

concentration in the methylene blue solution are not limited to these, but may be adjusted as appropriate in response to conditions such as the amount of hydrogen which would be dissolved in the water to be tested.

Next, 50 mL of one-fortieth strength Pt standard solution prepared as described above and 50 mL of each of the two types of different concentrations of methylene blue solution are respectively collected in individual degasification bottles, these are subjected three times to a process that includes 10 minutes of degasification using a vacuum pump followed by 10 minutes of nitrogen gas inclusion, so as to prepare the methylene blue solution and one-fortieth strength Pt standard solution that has undergone nitrogen gas replacement. This process aims to remove other gaseous components besides nitrogen (inert gas) in each of the solutions.

Next, 200 mL of test water is poured into an acrylic, gas-impermeable tester together with a magnet stirrer. This tester has been created for this testing and has a structure whereby the bottom is formed by attaching a round acrylic plate to one end along the length of a hollow, cylinder-shaped, acrylic tube, and the open end has a structure that has a pusher configured with a round plate having a diameter that is slightly smaller than the inner diameter of this tube so as to seal in a piston-like manner allowing movement along the length of the tube. A sealing ring made of a material such as silicon rubber is attached to the pusher so as to seal its entire surroundings. On the inside wall of this tester, a solution injection part configured with a hollow, cylinder-shaped, acrylic tube directed so as to radiate out towards the outside wall is provided in this tester to allow injection of MB solution or one-fortieth strength Pt standard solution separated from the outside environment into the test water holding compartment demarcated by the bottom surface, side wall, and pusher of this tester. In addition, a removable rubber stopper is provided for this solution injection part to allow syringe needle insertion. When pouring the test water into the test water holding compartment of the tester configured in this manner, the test water is softly pumped while the pusher is removed from the tester and then the pusher is attached to prevent vapor from forming inside the test water holding compartment. This allows the test water inside the test water holding compartment of the tester to be sealed in a condition separate from the outside environment. In addition, when the one-fortieth strength Pt standard solution or MB solution is poured into the test water holding compartment of the tester, such solution is collected through suction to prevent vapor from developing inside the syringe. The solution is gently injected by inserting

the needle of the syringe into the rubber stopper equipped with a solution injection part and pushing the piston of the syringe. It should be noted that the tester disclosed here is merely an example. Other appropriate vessels may be used as long as they meet conditions including: 1) the tester is made of a gas-impermeable material, and does not

5   occlude hydrogen (for example, stainless steel is not a suitable material for the tester since it occludes hydrogen, the target to be measured, although it is gas-impermeable); 2) test water holding compartment can be isolated from outside environment; 3) volume of test water holding compartment is adjustable; 4) test water holding compartment is air-tight and water-tight; 5) one-fortieth strength Pt standard solution and MB solution

10   may be poured in while the test water holding compartment is isolated from the outside environment; and 6) the stirrer is moveable.

    Next, the tester containing the test water described above is placed bottom down on a magnetic stirring table and stirring with the stirrer is begun.

    Next, 1 mL of the one-fortieth strength Pt standard solution that has been

15   subjected to the nitrogen gas replacement described above is injected to the test water holding compartment using a syringe and is then sufficiently stirred and mixed.

    Next, a predetermined density of methylene blue solution that has undergone the above-mentioned nitrogen gas replacement is injected a little bit at a time using a syringe while visually observing the color change of the test water. Here, if the

20   dissolved hydrogen concentration of the test water is greater than the amount of methylene blue poured in, then the methylene blue is reduced and becomes colorless. However, as the amount of methylene blue solution poured in gradually increases, the added methylene blue and the dissolved hydrogen of the test water counteract each other, and in time the change in the methylene blue from blue to colorless can no longer

25   be observed. Making this point the end point, the concentration of dissolved hydrogen DH in the test water can be found from the methylene blue concentration of the methylene blue solution and the total amount of methylene blue solution added.

(E) Finding the effective concentration of dissolved hydrogen

    In the following, the meaning of the 'effective dissolved hydrogen concentration

30   DH' is explained while showing the formula for finding the effective dissolved hydrogen concentration DH in the test water from the concentration and total added amount of the methylene blue solution added to the test water and the process of deriving the formula.

    To begin with, in the following description, the volume of water to be tested is

given as 200 mL and the methylene blue volume mole concentration of the methylene blue solution to be added to the test water is given as N(μmol/L). Moreover, given that the total amount of methylene blue solution added to reach the end point is A (mL), the total added amount of methylene molecules B(mol) becomes

5      $B = N \cdot A(\mu mol/L \times mL)$

        $= N \cdot A(m\mu mol)$ ...(Equation 1)

Here, given that the chemical formula of the methylene blue molecule is given as MBCl, and the chemical formula of the hydrogen molecule as H_2, the reaction in the solution between the hydrogen molecule activated by the Pt colloid and the methylene blue

10   molecule may be expressed with the following Reaction Formula 1.

        $H_2 + MBCl \square HCl + MBH$ ...(Reaction Formula 1)

        Here, HCl is hydrochloric acid, and MBH is reduced methylene blue. According to Reaction Formula 1, 1 mole of hydrogen molecules and 1 mole of methylene blue molecules react and generate 1 mole of reduced methylene blue molecules. In

15   order to explain the reception of electrons, the reaction formula may be written divided into two half equations as follows:

        $H_2 \square H^+ + (H^+ + 2e^-)$ ...(Half Reaction Formula 1)

        $MB^+ + (H^+ + 2e^-) \square MBH$ ...(Half Reaction Formula 2)

        Half Reaction Formula 1 means that the 1 mole of hydrogen molecules releases

20   2 moles of electrons, and half equation 2 means that the 1 mole of methylene blue cations, or 1 mole of methylene blue molecules accepts 2 moles of electrons. Here, 1 mole of hydrogen molecules is equivalent to 2 grams since 2 moles of electrons are released. Meanwhile, 1 mole of methylene blue cations, or 1 mole of methylene blue molecules is equivalent to 2 grams since 2 moles of electrons are accepted. As a result,

25   since the gram equivalence of both the hydrogen molecule and the methylene blue cation or the methylene blue molecule is 2, the hydrogen molecule and the methylene blue molecule react at a rate of 1 to 1 in terms of the mole ratio.

        In keeping with this, the total amount of methylene blue B added to the test water described above is also the amount of hydrogen molecules consumed.

30   Accordingly, given a total amount of hydrogen molecules to be measured as C(mμmol), the following may be obtained from Equation 1:

        $C = B = N \cdot A(m\mu mol)$ ...(Equation 2)

Moreover, if the volume of test water is 200 mL and the value of the effective hydrogen molecule mole concentration by volume H_2 (mol/L) of the test water is the mole count

C(mol) divided by volume (mL), then

H_2 (mol/L) = C /200 (mµmol/mL)

$$= C/200 \text{ (µmol/L)} \quad ...(\text{Equation 3})$$

Moreover, in the case of exchanging this unit with mass concentration (g/L), given the corresponding mass concentration of hydrogen molecules as D, from the proportional expression relating to the hydrogen molecule H_2:

1 mol /2 g = H_2 (µmol/L) /D   ... (Equation 4)

If this Equation 4 is substituted by Equation 3, then

$$D = 2 \cdot C/200 \text{ (µg/L)}$$

$$= C/100 \text{ (µg/L)} \quad ...(\text{Equation 5})$$

This is the mass concentration of effective hydrogen molecules included in 200 mL of test water. It should be noted that the above-mentioned effective hydrogen molecule mass concentration D is of the microgram order, however, both the numerator and the denominator may be multiplied by 1000 to give:

$$D = C \cdot 1000/100 \cdot 1000 \text{ (µg/L)}$$

$$= C \cdot 10^{-5} \text{ (mg/L)} \quad ...(\text{Equation 6})$$

Then from the relationship in Equation 2, since the hydrogen molecule mole count C of Equation 6 may be replaced with the total amount of methylene blue B, it may be established that:

$$D = N \cdot A \text{ (mµmol)} \cdot 10^{-5} \text{ (mg/L)} \quad ...(\text{Equation 7})$$

From this Equation 7, it may be understood that the effective hydrogen molecule mass concentration D (mg/L) included in the test water may be found by multiplying the methylene blue mole concentration by volume (µmol/L) by the total amount (mL) of methylene blue solution added to reach the end point.

However, the test water not only includes the hydrogen molecules (hydrogen gas) tested in the quantitative analysis here, but also includes various types of ions, oxygen molecules (oxygen gas), carbon dioxide (carbon dioxide gas), and the like. Of these, to give exemplary substance names involved in the oxidation/reduction reaction occurring in the test water, oxygen molecules, hypochlorite, hypochlorous acid, etc. may be given besides the hydrogen molecules. Including the oxidation/reduction reaction, such oxygen molecules, etc. normally act as the main oxidizing agent, and except for certain special cases, do not act as the reducing agent. In particular, in the test where methylene blue such as that described here is reduced, the oxygen molecules, etc. act as an oxidizing agent, and instead of reducing the methylene blue, act to oxidize the

reduced methylene blue changing it to oxidized methylene blue. In other words, even if the methylene blue reduced by the activation of the molecular hydrogen either remains reduced methylene blue and clear, or remains a white deposit, in the case where it exists together with the oxygen molecule, etc., the reduced methylene blue ends up being oxidized again and returning to the original oxidized methylene blue. In addition, even if not through the methylene blue, since the activated hydrogen molecule and the oxygen molecule directly react and take an equivalent amount of the reducing power of the hydrogen molecule, this equivalent amount of methylene can no longer be reduced. In other words, as shown in FIGS. 21 and 22, in the case where the oxygen molecules, etc. also exist in the hydrogen-dissolved water, an amount of hydrogen molecules equivalent to these amounts is consumed, and the total amount of methylene blue added until the equivalence point also becomes reduced in accordance with the amount of oxide.

In light of this, it may be said that the dissolved hydrogen concentration measured through quantitative analysis using methylene blue is the effective dissolved hydrogen concentration minus that consumed by oxidizing agents such as dissolved oxygen.

(F) Disclosure of reference examples and working examples

(Reference Example 17)

Using alkali electrolyzed water that has been subjected to continuous electrolysis processing using electrolysis conditions of electrolysis range '4' at normal water level with a 'Mini Water' electrolyzed water generation apparatus (equipped with an active charcoal filter) manufactured by MiZ Co., Ltd. as the test water, 1 mL of one-fortieth strength Pt standard solution that has been subjected to the nitrogen gas replacement described above is injected into the test water holding compartment using a syringe. This is then sufficiently stirred and mixed, and thereafter while visually observing the color change of the test water, a 1 g/L concentration (mole concentration by volume: 2677.4 µM) of methylene blue solution is added a little at a time to this test water using a syringe. The total amount of methylene blue injected until reaching the end point was 1 mL, and the effective value of dissolved hydrogen concentration DH found by substituting each value into Equation 7 was 0.03 (mg/L). For the test water according to this Working Example 17, the pH, oxidation/reduction potential ORP (mV), electric conductance EC (mS/m), water temperature T (°C), dissolved oxygen concentration DO (mg/L), effective value of dissolved hydrogen concentration DH (mg/L),

and the measured dissolved hydrogen concentration DH (mg/L) found by substituting each value into Equation 7 are shown in Table 3, and the measured value and the effective value of DH are shown in FIG. 23. It should be noted that the types of instruments used to measure each physical property value are the same as those described above.

(Reference Example 18)

Using test water that consists of purified water processed by passing Fujisawa city water through an ion exchange column manufactured by Organo Corporation, boiled, and then subjected to hydrogen gas bubbling processing while allowing the temperature to cool to 20°C, 1 mL of one-fortieth strength Pt standard solution that has undergone the nitrogen gas replacement described above is injected into 200 mL of this test water in a test water holding compartment using a syringe. This is then sufficiently stirred and mixed, and thereafter while visually observing the color change of the test water, a 10 g/L concentration (mole concentration by volume: 26773.8 µM) of methylene blue solution is injected a little bit at a time into the test water using a syringe. The total amount of methylene blue solution injected until reaching the end point was 6.2 mL, and the effective value of dissolved hydrogen concentration DH found by substituting each value into Equation 7 was 1.66 (mg/L). Each physical property value of the test water according to this Reference Example 18 is shown in Table 3, and the actual measured value and effective value of the dissolved hydrogen concentration DH are shown in FIG. 23.

(Working Example 20)

Using electrolyzed water as test water, which is base water 6.86 of the above-mentioned sample i that has been subjected to electrolysis processing using a continuous flow method under conditions of a 1 L/min flow and 5A constant current, 1 mL of one-fortieth strength Pt standard solution that has undergone the nitrogen gas replacement described above is injected to 200 mL of this test water in a test water holding compartment using a syringe. This is then sufficiently stirred and mixed, and thereafter while visually observing the color change of the test water, a 10 g/L concentration (mole concentration by volume: 26773.8 µM) of methylene blue solution is injected a little bit at a time into the test water using a syringe. The total amount of methylene blue solution injected until reaching the end point was 5.9 mL, and the effective value of dissolved hydrogen concentration DH found by substituting each value into Equation 7 was 1.58 (mg/L). Each physical property value of the test water

according to this Working Example 20 is shown in Table 3, and the actual measured value and effective value of the dissolved hydrogen concentration DH are shown in FIG. 23.

(Working Example 21)

5   Using electrolyzed water as test water, which is base water 9.18 of the above-mentioned sample v that has been subjected to electrolysis processing using a continuous flow method under conditions of a 1 L/min flow and 5A constant current, 1 mL of one-fortieth strength Pt standard solution that has undergone the nitrogen gas replacement described above is injected to 200 mL of this test water in a test water

10   holding compartment using a syringe. This is then sufficiently stirred and mixed, and thereafter while visually observing the color change of the test water, a 10 g/L concentration (mole concentration by volume: 26773.8 µM) of methylene blue solution is injected a little bit at a time into the test water using a syringe. The total amount of methylene blue solution injected until reaching the end point was 5.0 mL, and the

15   effective value of dissolved hydrogen concentration DH found by substituting each value into Equation 7 was 1.34 (mg/L). Each physical property value of the test water according to this Working Example 21 is shown in Table 3, and the actual measured value and effective value of the dissolved hydrogen concentration DH are shown in FIG. 23.

20   (Working Example 22)

Using electrolyzed water as test water, which is a pH buffer solution of standard buffer solution 4.01 (phthalate solution) manufactured by Wako Pure Chemical Industries, Ltd. diluted to one-tenth strength with purified water that has been subjected to electrolysis processing using a continuous flow method under

25   conditions of a 1 L/min flow and 5A constant current, 1 mL of one-fortieth strength Pt standard solution that has undergone the nitrogen gas replacement described above is injected into 200 mL of this test water in a test water holding compartment using a syringe. This is then sufficiently stirred and mixed, and thereafter while visually observing the color change of the test water, a 10 g/L concentration (mole concentration

30   by volume: 26773.8 µM) of methylene blue solution is injected a little bit at a time into the test water using a syringe. The total amount of methylene blue solution injected until reaching the end point was 6.3 mL, and the effective value of dissolved hydrogen concentration DH found by substituting each value into Equation 7 was 1.69 (mg/L). Each physical property value of the test water according to this Working Example 22 is

shown in Table 3, and the actual measured value and effective value of the dissolved hydrogen concentration DH are shown in FIG. 23.

(Working Example 23)

Using circulating electrolyzed water as test water, which is base water 6.86 of the above-mentioned sample i that has been subjected to electrolysis processing using a continuous flow circulating method (volume of circulatory water: 0.8 liters) for three minutes under conditions of a 1 L/min flow and 5A constant current, 1 mL of one-fortieth strength Pt standard solution that has undergone the nitrogen gas replacement described above is injected to 200 mL of this test water in a test water holding compartment using a syringe. This is then sufficiently stirred and mixed, and thereafter while visually observing the color change of the test water, a 10 g/L concentration (mole concentration by volume: 26773.8 µM) of methylene blue solution is injected a little bit at a time into the test water using a syringe. The total amount of methylene blue solution injected until reaching the end point was 9.6 mL, and the effective value of dissolved hydrogen concentration DH found by substituting each value into Equation 7 was 2.57 (mg/L). Each physical property value of the test water according to this Working Example 23 is shown in Table 3, and the actual measured value and effective value of the dissolved hydrogen concentration DH are shown in FIG. 23.

(Working Example 24)

Using circulating electrolyzed water as test water, which is base water 9.18 of the above-mentioned sample v that has been subjected to electrolysis processing using a continuous flow circulating method (volume of circulatory water: 0.8 liters) for three minutes under conditions of a 1 L/min flow and 5A constant current, 1 mL of one-fortieth strength Pt standard solution that has undergone the nitrogen gas replacement described above is injected to 200 mL of this test water in a test water holding compartment using a syringe. This is then sufficiently stirred and mixed, and thereafter while visually observing the color change of the test water, a 10 g/L concentration (mole concentration by volume: 26773.8 µM) of methylene blue solution is injected a little bit at a time into the test water using a syringe. The total amount of methylene blue solution injected until reaching the end point was 12.3 mL, and the effective value of dissolved hydrogen concentration DH found by substituting each value into Equation 7 was 3.29 (mg/L). Each physical property value of the test water according to this Working Example 24 is shown in Table 3, and the actual measured

value and effective value of the dissolved hydrogen concentration DH are shown in FIG. 23.

(Working Example 25)

Using circulating electrolyzed water as test water, which is the same pH buffer solution as Working Example 22 that has been subjected to electrolysis processing using a continuous flow circulating method (volume of circulatory water: 0.8 liters) for three minutes under conditions of a 1 L/min flow and 5A constant current, 1 mL of one-fortieth strength Pt standard solution that has undergone the nitrogen gas replacement described above is injected to 200 mL of this test water in a test water holding compartment using a syringe. This is then sufficiently stirred and mixed, and thereafter while visually observing the color change of the test water, a 10 g/L concentration (mole concentration by volume: 26773.8 μM) of methylene blue solution is injected a little bit at a time into the test water using a syringe. The total amount of methylene blue solution injected until reaching the end point was 12.4 mL, and the effective value of dissolved hydrogen concentration DH found by substituting each value into Equation 7 was 3.32 (mg/L). Each physical property value of the test water according to this Working Example 25 is shown in Table 3, and the actual measured value and effective value of the dissolved hydrogen concentration DH are shown in FIG. 23.

[Table 3]

★

(G) Examination of working examples

According to Table 3 and FIG. 23, it may be understood that there is commensurate correlation between the actual measured value and the effective value of the dissolved hydrogen concentration DH since when the actual measured value is high, the effective value grows higher in response thereto. In addition, compared to the dissolved hydrogen concentration DH effective value of Reference Example 17, the respective effective values of DH in Reference Example 18 and Working Examples 20 through 25 all showed high concentrations exceeding 1.3 (mg/L). In particular, while the molecular hydrogen saturated solvent concentration under normal temperature (20°C) and atmospheric pressure is approximately 1.6 (mg/L), which approaches that of water, the DH effective values of Working Examples 20 through 25 showed between 2.5 and 3.3 (mg/L), which are exceedingly high concentrations.

Therefore oxygen molecules may be thought of as being the main oxidizing

agent remaining in the test water since in the quantitative analysis testing of dissolved-hydrogen concentrations performed herein, water that was pre-treated with activated charcoal was used (without adding a reducing agent) in all cases to scavenge the chlorine-based oxidizers such as hypochlorous acid. It should be noted that even if the oxygen molecules are temporarily scavenged with the activated charcoal, as long as there is no sort of reducing agent used, it is difficult to scavenge with only activated charcoal because oxygen quickly blends back into the water as soon as the test water hits the outside air.

Nevertheless, with the premise that the proposed antioxidation method according the present invention is used, the fact that the concentration of oxidizing material such as dissolved oxygen may be kept as low as possible while also making the dissolved hydrogen concentration as high as possible with a reducing potential water generation apparatus such as that developed by the applicants herein is important when anticipating expression of reduction activity and antioxidation activity that may be derived from the antioxidant-functioning water according to the combination of catalysts and hydrogen-dissolved water according to the present invention.

Therefore, in attempt to define the dissolved hydrogen water according to the present invention from the standpoint of the effective value of dissolved hydrogen concentration DH found using dissolved hydrogen concentration quantitative analysis that uses oxidation/reduction pigment according to the present invention, it is preferable that the DH effective value be greater than the saturation concentration under atmospheric pressure (for example, 1.6 or greater under an atmospheric pressure of 1, 3.2 or greater under an atmospheric pressure of 2, 4.8 or greater under an atmospheric pressure of 3, and the like). Furthermore, when the atmospheric pressure is 1, as the dissolved hydrogen concentration DH effective value becomes higher preference increases, such as in the following order: 1.7 or greater, 1.8 or greater, 1.9 or greater, 2.0 or greater, 2.1 or greater, 2.2 or greater, 2.3 or greater, 2.4 or greater, 2.5 or greater, 2.6 or greater, 2.7 or greater, 2.8 or greater, 2.9 or greater, 3.0 or greater, 3.1 or greater, 3.2 or greater, and 3.3 or greater (all units are mg/L). This is because reduction activity and antioxidation activity derived from the antioxidant-functioning water according to the combination of catalysts and hydrogen-dissolved water according to the present invention may be anticipated with higher levels.

This information proposes a new quantitative analysis method of hydrogen concentration for hydrogen-dissolved water including electrolyzed water as well as a

new measure of the explicit antioxidation power held by this water. In addition, with dissolved hydrogen concentration measurement using an existing dissolved hydrogen meter, handling and measurement procedures are complicated, in terms of measurement precision such measurement is also incapable of providing sufficient satisfaction, and furthermore, related costs are extremely high. However, with the dissolved hydrogen concentration quantitative analysis method according to the present invention that uses oxidation/reduction pigment, handling and measurement procedures is relatively simple, and if the oxidation material included in the test water is scavenged, high precision is realized in terms of accuracy because it is based on the principle of performing direct, quantitative analysis through the chemical reaction of the number of molecules of molecular hydrogen with the oxidation/reduction pigment, and moreover, the related costs are extremely low.

Radical scavenging activity evaluation test using epinephrine oxidation method in XOD experiment system

Next, a radical scavenging activity evaluation test using an epinephrine oxidation method in an XOD experiment system is described in the following (A). In addition, radical scavenging activity expressed in antioxidant-functioning water (hereafter, may be referred to as 'AOW') according to the present invention when using this testing method is shown in the following (B) through both working examples and reference examples, respectively, where precious metal colloid catalysts (Pt colloid/ Pd colloid/ Pt and Pd mixed colloid) added into hydrogen-dissolved water as AOW are given as examples. It should be noted that the XOD experiment system is an experiment system that generates a superoxide anion radical (hereafter, may be simply referred to as ($\cdot O_2^-$)) by reducing one electron of oxygen using the electrons released when xanthine is oxidized through a process where xanthine oxidase (XOD), which is an organism enzyme, acts on xanthine in the oxygen-dissolved solution system.

(A) Radical scavenging activity evaluation test using epinephrine oxidation method in XOD experiment system

(A-1) Development of idea

The cytochrome c reduction method is known as a conventional free radical measurement method, as described in 'Free radical and medicine' (issued by Hirokawa Shoten ISBN 4-567-49380-X, p133-141).

With this cytochrome c reduction method (see FIG. 24), in the XOD experiment system, the reduction reaction of the oxidized cytochrome c (ferricytochrome c (Fe^{3+})

into oxidized cytochrome c (ferrocytochrome c (Fe^{2+}) by the assistance of the superoxide anion radical ($\cdot O_2^-$) being retarded by a test sample such as SOD or antioxidizing agent is observed through a change over time in the local maximum absorption (wavelength: 550 nm) of the oxidized cytochrome c using a spectrophotometer. This method utilizes

5   reactivity of ($\cdot O_2^-$) as a single-electron reducing agent.

To further explain this measurement principle in detail, in the cytochrome c reduction method, when a test sample such as an antioxidizing agent reduces and scavenges ($\cdot O_2^-$) prior to (or simultaneous to) the reduction reaction of the oxidized cytochrome c due to ($\cdot O_2^-$), the reduced amount of oxidized cytochrome c, namely, the

10   generated amount of reduced cytochrome c is controlled (either upward tendency is controlled, or downward tendency begins to show.) Then, the local maximum absorption (A550) of the reduced cytochrome c also decreases. To consider this with time, the local maximum absorption (A550) of the reduced cytochrome c per unit time decreases along with the scavenged amount of ($\cdot O_2^-$). By observing transition of this

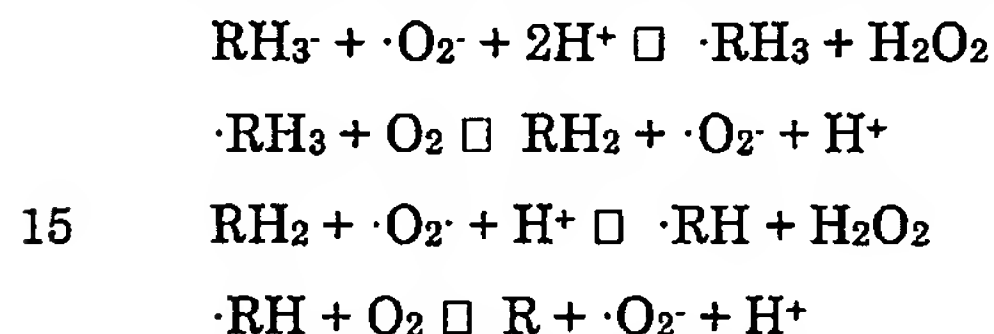15   local maximum absorption (A550) over time, SOD-like activity of the test sample can be measured.

However, when a test sample having remarkable reducing power such as ascorbic acid or the antioxidant-functioning water according to the present invention is used, there are problems with the SOD-like activity measurement system using the

20   above-mentioned conventional cytochrome c reduction method in that it cannot obtain highly precise measurement results, as well as it leads to opposite measurement results to the expected true results. This is because ($\cdot O_2^-$) is used as a reducing agent. In other words, AOW and ascorbic acid of the present invention reduces and scavenges ($\cdot O_2^-$), and even reduces the oxide cytochrome c. As a result, for example, ($\cdot O_2^-$) may be

25   completely reduced and scavenged, and the oxidized cytochrome c may also be reduced with its residual reducing power. Therefore, there has been a risk of not obtaining highly precise measurement results, as well as of leading to opposite measurement results, such as low SOD-like activity appearance, to the expected true results.

As a result of zealous research on the free radical reaction reagent suitable for

30   radical scavenging activity evaluation testing of antioxidant-functioning water according to the present invention, the inventers ended up wondering if oxidation of reduced epinephrine can be used.

When the reduced epinephrine is oxidized by the superoxide anion radical ($\cdot O_2^-$), it changes to red adrenochrome (oxidized epinephrine), and its local maximum

absorption (A480) increases. In this case, ($\cdot O_2^-$) acts as an oxidizing agent. It should be noted that the reduced epinephrine is difficult to be oxidized by a normal oxygen molecular, and even if it is oxidized, it does not indicate a color change reaction to red. That is, it has been confirmed through experiment that the local maximum absorption

5   (A480) of the reduced epinephrine does not increase even in oxygen-dissolved solution system. This means that the reduced epinephrine is suitable as a free radical reaction reagent to distinguish a normal oxygen molecular (O_2) from a superoxide anion radical ($\cdot O_2^-$).

In the epinephrine oxidation method (see FIG. 25), to begin with, it is assumed

10   that a sufficient amount of ($\cdot O_2^-$) has been generated in the XOD experiment system. ($\cdot O_2^-$) generated in this manner changes the reduced epinephrine to oxidized epinephrine in conformity with the following chain reaction.

$$RH_3^- + \cdot O_2^- + 2H^+ \square \cdot RH_3 + H_2O_2$$
$$\cdot RH_3 + O_2 \square RH_2 + \cdot O_2^- + H^+$$

15    $$RH_2 + \cdot O_2^- + H^+ \square \cdot RH + H_2O_2$$
$$\cdot RH + O_2 \square R + \cdot O_2^- + H^+$$

Where (RH_3^-) is reduced epinephrine, and (R) is oxidized epinephrine (adrenochrome). While the reactivity between the reduced epinephrine and ($\cdot O_2^-$) is low in the vicinity of the physiological region in pH, the speed of a secondary reaction between the reduced

20   epinephrine and ($\cdot O_2^-$) increases if a large dosage of reduced epinephrine is administered. Therefore, with this epinephrine oxidation method, it is preferable that the mole concentration of reduced epinephrine be set to a high concentration of approximately 1 mM, for example. Furthermore, the reduced epinephrine tends to be easily oxidized by trace amounts of a transition metal such as an iron ion. In order to

25   avoid influence from the above-mentioned disturbances, it is necessary to include a chelating agent such as EDTA in the test water.

At this time, prior to (or simultaneous to) the oxidation reaction of reduced epinephrine due to ($\cdot O_2^-$), the oxidized amount of reduced epinephrine, that is, the generated amount of oxidized epinephrine is being gradually reduced as a test sample

30   such as an antioxidizing agent reduces and scavenges ($\cdot O_2^-$). Then, the local maximum absorption (A480) of the oxidized epinephrine tends to rise gradually. To consider this with time, the local maximum absorption (A480) of the reduced epinephrine per unit time tends to rise gradually along with the scavenged amount of ($\cdot O_2^-$). Therefore, by observing transition of this local maximum absorption (A480) over time, SOD-like

activity (radical scavenging activity) of the test sample can be measured.

More specifically, to consider a graph where the horizontal axis indicates elapsed time and the vertical axis indicates light absorbance (A480), the SOD-like activity can be represented by the amount of change (ΔA480) in the light absorbance (A480) per unit time (ΔT). In other words, the tangent slope (ΔA/ΔT) in the graph represents the SOD-like activity. Therefore, regarding the SOD-like activity graph of a test sample, it can be determined that when a positive slope (continuously growing characteristic) is large, the SOD-like activity is low; whereas, when the positive slope is small, the SOD-like activity is high. On the other hand, it can be determined that when a negative slope (continuously dropping characteristic) is large, the SOD-like activity is high; whereas, the negative slope is small, the SOD-like activity is low. However, comparing the case of a small positive slope to the case of a small negative slope from the standpoint of high or low radical scavenging activity shows that the latter has higher radical scavenging activity than the former.

This is a measurement principle of the radical scavenging activity (SOD-like activity) using the epinephrine oxidation method.

(A-2) Reagents to be used

Reagents to be used for radical scavenging activity (SOD-like activity) testing using the epinephrine oxidation method are given below.

(1) Dulbecco's phosphoric acid buffer physiological salt (PBS) manufactured by Wako Pure Chemical Industries, Ltd.

(2) Xanthine (2,6-dioxopurine) manufactured by Wako Pure Chemical Industries, Ltd.

(3) Xanthineoxidase suspension (derived from buttermilk) manufactured by Wako Pure Chemical Industries, Ltd.

(4) EDTA-2Na 2 hydrate manufactured by Dojindo Laboratories, and sold by Wako Pure Chemical Industries, Ltd.

(5) 1 mol/L sodium hydroxide solution manufactured by Wako Pure Chemical Industries, Ltd.

(6) L(+)-ascorbic acid manufactured by Wako Pure Chemical Industries, Ltd.

(7) (±)epinephrine (dl-epinephrine) (dl-adrenaline) manufactured by Wako Pure Chemical Industries, Ltd.

(A-3) Reagent preparation method

Preparation methods for the respective reagents given in the above section (A-2) are described below.

(1) Preparation of PBS buffer stock solution containing EDTA

Two packages of 500 mL of Dulbecco's phosphoric acid buffer physiological salt (hereafter, referred to as 'PBS') are dissolved into 100 mL of distilled water, and is then divided into two equal parts. 0.19 g of EDTA-2Na is dissolved into one of the 50 mL of solution. This shall be an EDTA stock solution. 0.5 mL of this EDTA stock solution and 49.5 mL of a solution containing no EDTA are collected, respectively, and then are mixed together.

The mixed solution obtained here is a PBS buffer stock solution containing EDTA (1.9 mg). This is used diluted to one-tenth strength PBS concentration. If many metal ion species are contained in test water, the EDTA stock solution may be diluted to one-tenth strength. In this case, since the EDTA concentration is high, almost all of metal ions, which are factors that degrade measurement accuracy, can be removed. It should be noted that the PBS buffer stock solution containing EDTA is prepared in order to fix the pH of the solution to the vicinity of approximately 7.4, which falls within the physiological region of solution characteristics, and prevent measurement accuracy from being degraded due to metal ions.

(2) Preparation of 1.5 mM of xanthine solution

$0.228 \approx 0.23$ g of xanthine and 80 drops of 1 (mol/L) sodium hydroxide are added to 350 mL of distilled water, and the xanthine is dissolved. 35 mL of that solution is collected, and the PBS buffer stock solution containing EDTA is added to make 100 mL of solution.

(3) Preparation of xanthineoxidase solution

Xanthineoxidase suspension is diluted with the PBS buffer stock solution containing EDTA to one-one hundredth strength. This must be prepared for each experiment.

(4) Preparation of epinephrine solution

100 mL of distilled water is poured into a vial together with a stirrer, a rubber stopper is inserted, and an injection needle for exhaustion and an injection needle connected to a nitrogen gas cylinder are inserted into the rubber stopper. Under this condition, nitrogen gas is included into the distilled water while vigorously stirring the vial containing the distilled water, which is placed on the stirring table, and replacement in the distilled water is made with nitrogen gas. This nitrogen gas replacement treatment is carried out for 30 minutes. 0.15 g of epinephrine is then poured into the vial, the stopper is inserted, and nitrogen gas is included while softly

stirring with the stirrer. This nitrogen gas replacement treatment continues until the experiment ends. This is the preparation of the epinephrine solution. Tips on the above-mentioned preparation are to stir vigorously before epinephrine is added, and to stir softly after epinephrine is added.

5   (A-4) Testing procedures

In the conventional XOD experiment system, all reagents and a test solution are added into a cell sequentially, and xanthineoxidase (XOD) is added last. While the reaction has begun with the addition of this XOD, measurement using a spectrophotometer has also begun.

10   However, with the conventional method, when the test solution is added, $(\cdot O_2^-)$ has not been sufficiently generated yet. Therefore, at this time, oxygen molecules, which are materials for generating $(\cdot O_2^-)$, exist without change. In other words, since the generated amount of $(\cdot O_2^-)$ increases as the oxygen molecules change to $(\cdot O_2^-)$ with time after XOD is added, an appropriate amount of $(\cdot O_2^-)$ cannot be obtained immediately after the test water is added. Furthermore, since the amount of dissolved

15   oxygen, which is a material for generating $(\cdot O_2^-)$, is not understood clearly with the conventional method, it is not clear whether or not the same amount of $(\cdot O_2^-)$ is generated every time.

Through consideration of the above, the inventors have come to believe that it is desirable to begin by adding the PBS buffer stock solution containing EDTA, the

20   xanthineoxidase solution, and the distilled water for supplying oxygen to a cell and mixing together. After a specified period has passed, that is, after an appropriate amount of $(\cdot O_2^-)$ has been generated, the test water and the epinephrine solution are added and measurement is begun.

25   Therefore, in this epinephrine oxidation method, each reagent solution or the test water is poured into a cell sequentially according to the following procedures. In addition, a waiting period is included according to need. It should be noted that the cell has 3 mL of capacity of which each of the test water and the distilled water for supplying oxygen occupy approximately 1/3, respectively, and the remaining

30   approximately 1/3 is occupied by a xanthineoxidase solution, an epinephrine solution, and a xanthine solution such as a pH buffer solution containing EDTA.

(1) Add 300 μL of PBS buffer stock solution containing EDTA.

(2) Add 300 μL of xanthine solution.

(3) Add 900 μL (approximately 1/3 of the cell capacity) of distilled water for supplying

oxygen.

(4) Add 100 μL of xanthineoxidase solution.

(5) Wait 5 minutes to generate an appropriate amount of ($\cdot$O$_2$$\cdot$).

(6) Add 1 mL (approximately 1/3 of the cell capacity) of test water (fluid).

5   (7) Add 400 μL of epinephrine solution.

(8) Immediately start measurement of change in light absorbance (A480) over time using a spectrophotometer.

(9) Wait 140 seconds to level out the differences in the local concentration gradient of reagents within the cell.

10   In other words, measurement data taken within 140 seconds from the start of measurement is excluded as a rule from a radical scavenging activity characteristic graph to be described later.   The period for observing the change over time is set to 15 minutes except in the 140 second waiting period described in step (9).   This is because a clear difference in the radical scavenging activity between platinum and palladium

15   may not be recognized with an observation period of around 5 to 10 minutes, for example.

It should be noted that it has been found through the experiment that an indication of radical scavenging activity can be found through the conventional method where all reagents are collectively added and xanthineoxidase is added last, then

20   beginning the reaction and measurement.   However, it is preferable that test water be added after an appropriate amount of ($\cdot$O$_2$$\cdot$) has been generated in the XOD experiment system, and a clearer indication of the actual radical scavenging activity may be found. Furthermore, with the conventional method, since it tends to take a relatively long time to generate an appropriate amount of ($\cdot$O$_2$$\cdot$) depending on the production lot of the

25   xanthineoxidase, it is difficult to grasp the radical scavenging activity of the test water or the like in a short period of time.   In particular, the conventional method has poor accuracy for the usage of finding the difference in catalytic activity between platinum and palladium.   The timing of adding each reagent and of waiting should be specified as described above including such above-mentioned practical factors.

30   (B) Disclosure of working examples and reference examples according to radical scavenging activity evaluation testing by epinephrine oxidation method in XOD experiment system

(Reference Example 19)

Radical scavenging activity measurement data obtained in conformity with the

testing procedures described in (A-4) when distilled water (manufactured by Wako Pure Chemical Industries, Ltd.) is employed as test water is given as Reference Example 19. It should be noted that under the testing condition where xanthineoxidase with different production lots are used, the radical scavenging activity measurement data of this Reference Example 19 may have slightly different radical scavenging activity characteristics depending on the production lots.

(Reference Example 20)

Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Reference Example 19 when hydrogen gas-replaced distilled water (manufactured by Wako Pure Chemical Industries, Ltd.) is employed as test water is given as Reference Example 20. It should be noted that, as with Reference Example 19, under the testing condition where xanthineoxidase with different production lots are used, the radical scavenging activity measurement data of this Reference Example 20 may have slightly different radical scavenging activity characteristics depending on the production lots.

(Working Example 26)

Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Reference Example 19 when antioxidant-functioning water (AOW) is used as test water, which is obtained by adding to distilled water (manufactured by Wako Pure Chemical Industries, Ltd., hereafter, the same) an amount of Pt standard solution described in Working Examples 3 through 5 sufficient to give a Pt colloid concentration of 48 µg/L, and then subjecting it to hydrogen gas replacement, is given as Working Example 26.

(Working Example 27)

Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Working Example 26 when AOW is used as test water, which is obtained by adding to distilled water an amount of the same Pt standard solution as that of Working Example 26 sufficient to give a Pt colloid concentration of 96 µg/L, and then subjecting it to hydrogen gas replacement, is given as Working Example 27.

(Working Example 28)

Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Working Example 26 when AOW is used as test water, which is obtained by adding to distilled water an amount of the same Pt standard

solution as that of Working Example 26 sufficient to give a Pt colloid concentration of 192 µg/L, and then subjecting it to hydrogen gas replacement, is given as Working Example 28.

(Working Example 29)

5   Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Working Example 26 when AOW is used as test water, which is obtained by adding to distilled water an amount of the same Pt standard solution as that of Working Example 26 sufficient to give a Pt colloid concentration of 384 µg/L, and then subjecting it to hydrogen gas replacement, is given as Working

10   Example 28.

(Working Example 30)

        Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Working Example 26 when AOW is used as test water, which is obtained by adding to distilled water an amount of the same Pt standard

15   solution as that of Working Example 26 sufficient to give a Pt colloid concentration of 768 µg/L, and then subjecting it to hydrogen gas replacement, is given as Working Example 30.

(Working Example 31)

        Radical scavenging activity measurement data obtained in conformity with the

20   same testing procedures as those of Reference Example 19 when antioxidant-functioning water (AOW) is used as test water, which is obtained by adding to distilled water an amount of Pd standard solution described in Working Examples 6 through 8 sufficient to give a Pd colloid concentration of 48 µg/L, and then subjecting it to hydrogen gas replacement, is given as Working Example 31.

25   (Working Example 32)

        Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Working Example 31 when AOW is used as test water, which is obtained by adding to distilled water an amount of the same Pd standard solution as that of Working Example 31 sufficient to give a Pd colloid

30   concentration of 96 µg/L, and then subjecting it to hydrogen gas replacement, is given as Working Example 32.

(Working Example 33)

        Radical scavenging activity measurement data obtained in conformity with the same testing procedures as that of Working Example 31 when AOW is used as test

water, which is obtained by adding to distilled water the same Pd standard solution as that of Working Example 31 sufficient to give a Pd colloid concentration of 192 µg/L, and then subjecting it to hydrogen gas replacement, is given as Working Example 33.

(Working Example 34)

5   Radical scavenging activity measurement data obtained in conformity with the same testing procedures as that of Working Example 31 when AOW is used as test water, which is obtained by adding to distilled water the same Pd standard solution as that of Working Example 31 sufficient to give a Pd colloid concentration of 384 µg/L, and then subjecting it to hydrogen gas replacement, is given as Working Example 34.

10   (Working Example 35)

Radical scavenging activity measurement data obtained in conformity with the same testing procedures as that of Working Example 31 when AOW is used as test water, which is obtained by adding to distilled water the same Pd standard solution as that of Working Example 31 sufficient to give a Pd colloid concentration of 768 µg/L, and

15   then subjecting it to hydrogen gas replacement, is given as Working Example 35.

(Working Example 36)

Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Reference Example 19 when AOW is used as test water, which is obtained by adding to distilled water an amount of platinum colloid

20   solution that has approximately a particle size distribution of 1 to 2 nm and has been manufactured by the inventors based on the above-described thesis 'Fabrication and Use of Pt Colloids (Pt koroido no tsukurikata to tsukaikata)' written by Mr. NANBA, et al. sufficient to give a Pt colloid concentration of 66 µg/L, and then subjecting it to hydrogen gas replacement, is given as Working Example 36.

25   (Working Example 37)

Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Working Example 36 when AOW is used as test water, which is obtained by adding to distilled water an amount of the same platinum colloid solution as that of Working Example 36 sufficient to give a Pt colloid

30   concentration of 96 µg/L, and then subjecting it to hydrogen gas replacement, is given as Working Example 37.

(Working Example 38)

Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Working Example 36 when AOW is used as test

water, which is obtained by adding to distilled water an amount of the same platinum colloid solution as that of Working Example 36 sufficient to give a Pt colloid concentration of 144 μg/L, and then subjecting it to hydrogen gas replacement, is given as Working Example 38.

5   (Working Example 39)

Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Working Example 36 when AOW is used as test water, which is obtained by adding to distilled water an amount of the same platinum colloid solution as that of Working Example 36 sufficient to give a Pt colloid

10   concentration of 192 μg/L, and then subjecting it to hydrogen gas replacement, is given as Working Example 39.

(Working Example 40)

Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Working Example 36 when AOW is used as test

15   water, which is obtained by adding to distilled water an amount of the same platinum colloid solution as that of Working Example 36 sufficient to give a Pt colloid concentration of 384 μg/L, and then subjecting it to hydrogen gas replacement, is given as Working Example 40.

(Working Example 41)

20   Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Working Example 36 when AOW is used as test water, which is obtained by adding to distilled water an amount of the same platinum colloid solution as that of Working Example 36 sufficient to give a Pt colloid concentration of 768 μg/L, and then subjecting it to hydrogen gas replacement, is given

25   as Working Example 41.

(Working Example 42)

Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Reference Example 19 when AOW is used as test water, which is obtained by adding to distilled water a (Pt + Pd) colloid solution of 96

30   μg/L mixed and prepared with a mole ratio of 1 to 2 for the same Pt standard solution as that of Working Example 26 and the same Pd standard solution as that of Working Example 31, and then subjecting it to hydrogen gas replacement, is given as Working Example 42.

(Working Example 43)

Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Working Example 42 when AOW is used as test water, which is obtained by adding to distilled water an amount of the same mixed (Pt + Pd) colloid solution as that of Working Example 42 sufficient to give a concentration of

5   192 µg/L, and then subjecting it to hydrogen gas replacement, is given as Working Example 43.

(Working Example 44)

Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Working Example 42 when AOW is used as test

10   water, which is obtained by adding to distilled water an amount of the same mixed (Pt + Pd) colloid solution as that of Working Example 42 sufficient to give a concentration of 384 µg/L, and then subjecting it to hydrogen gas replacement, is given as Working Example 44.

(Working Example 45)

15   Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Working Example 42 when AOW is used as test water, which is obtained by adding to distilled water an amount of the same mixed (Pt + Pd) colloid solution as that of Working Example 42 sufficient to give a concentration of 768 µg/L, and then subjecting it to hydrogen gas replacement, is given as Working

20   Example 45.

(Working Example 46)

Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Reference Example 19 when AOW is used as test water, which is obtained by adding to distilled water a (Pt + Pd) colloid solution of 144

25   µg/L mixed and prepared with a mole ratio of 1 to 5 for the same Pt standard solution as that of Working Example 26 and the same Pd standard solution as that of Working Example 31, and then subjecting it to hydrogen gas replacement, is given as Working Example 46.

(Working Example 47)

30   Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Working Example 46 when AOW is used as test water, which is obtained by adding to distilled water a (Pt + Pd) colloid solution of 240 µg/L mixed and prepared with a mole ratio of 1 to 10 for the same Pt standard solution as that of Working Example 26 and the same Pd standard solution as that of Working

Example 31, and then subjecting it to hydrogen gas replacement, is given as Working Example 47.

(Working Example 48)

Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Working Example 46 when AOW is used as test water, which is obtained by adding to distilled water a (Pt + Pd) colloid solution of 336 µg/L mixed and prepared with a mole ratio of 1 to 15 for the same Pt standard solution as that of Working Example 26 and the same Pd standard solution as that of Working Example 31, and then subjecting it to hydrogen gas replacement, is given as Working Example 48.

(Working Example 49)

Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Working Example 46 when AOW is used as test water, which is obtained by adding to distilled water a (Pt + Pd) colloid solution of 432 µg/L mixed and prepared with a mole ratio of 1 to 20 for the same Pt standard solution as that of Working Example 26 and the same Pd standard solution as that of Working Example 31, and then subjecting it to hydrogen gas replacement, is given as Working Example 49.

(Working Example 50)

Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Working Example 46 when AOW is used as test water, which is obtained by adding to distilled water a (Pt + Pd) colloid solution of 528 µg/L mixed and prepared with a mole ratio of 1 to 25 for the same Pt standard solution as that of Working Example 26 and the same Pd standard solution as that of Working Example 31, and then subjecting it to hydrogen gas replacement, is given as Working Example 50.

(Reference Example 21)

Radical scavenging activity measurement data obtained in conformity with testing procedures, which are the testing procedures described in (A-4) with an altered part, when hydrogen gas-replaced distilled water is employed as test water is given as Reference Example 21. The alteration of the testing procedures is that instead of adding 300 µL of xanthine solution and 100 µL of xanthineoxidase solution to a tester cell, that is, instead of removing the ($\cdot O_2^-$) generation system, the amount of distilled water for supplying oxygen is increased from 900 µL to 1300 µL.

(Working Example 51)

Radical scavenging activity measurement data obtained in conformity with testing procedures, which are given by changing a part of the testing procedures described in (A-4), when AOW is used as test water, which is obtained by adding to distilled water a (Pt + Pd) colloid solution of 144 µg/L mixed and prepared with a mole ratio of 1 to 5 for the same Pt standard solution as that of Working Example 26 and the same Pd standard solution as that of Working Example 31, and then subjecting it to hydrogen gas replacement, is given as Working Example 51. The alteration of the testing procedures is the same as that of Reference Example 21.

(Working Example 52)

Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Working Example 51 when AOW is used as test water, which is obtained by adding to distilled water a (Pt + Pd) colloid solution of 240 µg/L mixed and prepared with a mole ratio of 1 to 10 for the same Pt standard solution as that of Working Example 26 and the same Pd standard solution as that of Working Example 31, and then subjecting it to hydrogen gas replacement, is given as Working Example 52.

(Working Example 53)

Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Working Example 51 when AOW is used as test water, which is obtained by adding to distilled water a (Pt + Pd) colloid solution of 336 µg/L mixed and prepared with a mole ratio of 1 to 15 for the same Pt standard solution as that of Working Example 26 and the same Pd standard solution as that of Working Example 31, and then subjecting it to hydrogen gas replacement, is given as Working Example 53.

(Working Example 54)

Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Working Example 51 when AOW is used as test water, which is obtained by adding to distilled water a (Pt + Pd) colloid solution of 432 µg/L mixed and prepared with a mole ratio of 1 to 20 for the same Pt standard solution as that of Working Example 26 and the same Pd standard solution as that of Working Example 31, and then subjecting it to hydrogen gas replacement, is given as Working Example 54.

(Working Example 55)

Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Working Example 51 when AOW is used as test water, which is obtained by adding to distilled water a (Pt + Pd) colloid solution of 528 µg/L mixed and prepared with a mole ratio of 1 to 25 for the same Pt standard solution as that of Working Example 26 and the same Pd standard solution as that of Working Example 31, and then subjecting it to hydrogen gas replacement, is given as Working Example 55.

(Reference Example 22)

Radical scavenging activity measurement data for test water obtained in conformity with testing procedures described in (A-4) when pH buffer solution (base water 6.86) is used as the test water, which is obtained by diluting standard buffer solution 6.86 (phosphate solution) manufactured by Wako Pure Chemical Industries, Ltd. to one-tenth strength with purified water, is given as Reference Example 22.

(Reference Example 23)

Radical scavenging activity measurement data for test water obtained in conformity with the same testing procedures as those of Reference Example 22 when catalyst-free electrolyzed water is used as the test water, which is the same base water 6.86 as that of Reference Example 22 that has been subjected to electrolysis processing using a continuous flow method under conditions of a 1.5 L/min flow and 5A constant current, is given as Reference Example 23.

(Working Example 56)

1 liter of the same base water 6.86 as that of Reference Example 22 has been collected, and the same Pt standard solution as that of Working Example 26 sufficient to give a concentration of 48 µg/L has been added to that water so as to prepare Pt colloid-containing base water 6.86. Radical scavenging activity measurement data for test water (AOW) obtained in conformity with the same testing procedures as those of Reference Example 22 when pre-electrolysis catalyst-added one-pass electrolyzed water is used as the test water (AOW), which is the Pt colloid-containing base water 6.86 prepared in this manner that has been subjected to electrolysis processing using a continuous flow method under the same conditions as those of Reference Example 23, is given as Working Example 56.

(Working Example 57)

1 liter of the same base water 6.86 as that of Reference Example 22 has been collected, and the same Pt standard solution as that of Working Example 26 sufficient to

give a concentration of 96 μg/L has been added to that water so as to prepare Pt colloid-containing base water 6.86. Radical scavenging activity measurement data for test water (AOW) obtained in conformity with the same testing procedures as those of Working Example 56 when pre-electrolysis catalyst-added one-pass electrolyzed water

5 is used as the test water (AOW), which is the Pt colloid-containing base water 6.86 prepared in the above-mentioned manner that has been subjected to electrolysis processing using a continuous flow method under the same conditions as those of Reference Example 23, is given as Working Example 57.

(Working Example 58)

10 1 liter of the same base water 6.86 as that of Reference Example 22 has been collected, and the same Pt standard solution as that of Working Example 26 sufficient to give a concentration of 192 μg/L has been added to that water so as to prepare Pt colloid-containing base water 6.86. Radical scavenging activity measurement data for test water (AOW) obtained in conformity with the same testing procedures as those of

15 Working Example 56 when pre-electrolysis catalyst-added one-pass electrolyzed water is used as the test water (AOW), which is the Pt colloid-containing base water 6.86 prepared in the above-mentioned manner that has been subjected to electrolysis processing using a continuous flow method under the same conditions as those of Reference Example 23, is given as Working Example 58.

20 (Working Example 59)

1 liter of the same base water 6.86 as that of Reference Example 22 has been collected, and the same Pt standard solution as that of Working Example 26 sufficient to give a concentration of 384 μg/L has been added to that water so as to prepare Pt colloid-containing base water 6.86. Radical scavenging activity measurement data for

25 test water (AOW) obtained in conformity with the same testing procedures as those of Working Example 56 when pre-electrolysis catalyst-added one-pass electrolyzed water is used as the test water (AOW), which is the Pt colloid-containing base water 6.86 prepared in the above-mentioned manner that has been subjected to electrolysis processing using a continuous flow method under the same conditions as those of

30 Reference Example 23, is given as Working Example 59.

(Working Example 60)

1 liter of the same base water 6.86 as that of Reference Example 22 has been collected, and the same Pt standard solution as that of Working Example 26 sufficient to give a concentration of 768 μg/L has been added to that water so as to prepare Pt

colloid-containing base water 6.86. Radical scavenging activity measurement data for test water (AOW) obtained in conformity with the same testing procedures as those of Working Example 56 when pre-electrolysis catalyst-added one-pass electrolyzed water is used as the test water (AOW), which is the Pt colloid-containing base water 6.86

5 prepared in the above-mentioned manner that has been subjected to electrolysis processing using a continuous flow method under the same conditions as those of Reference Example 23, is given as Working Example 60.

(Working Example 61)

1 liter of the same base water 6.86 as that of Reference Example 22 has been

10 collected, and the same Pd standard solution as that of Working Example 31 sufficient to give a concentration of 48 µg/L has been added to that water so as to prepare Pd colloid-containing base water 6.86. Radical scavenging activity measurement data for test water (AOW) obtained in conformity with the same testing procedures as those of Reference Example 22 when pre-electrolysis catalyst-added one-pass electrolyzed water

15 is used as the test water (AOW), which is the Pd colloid-containing base water 6.86 prepared in the above-mentioned manner that has been subjected to electrolysis processing using a continuous flow method under the same conditions as those of Reference Example 23, is given as Working Example 61.

(Working Example 62)

20 1 liter of the same base water 6.86 as that of Reference Example 22 has been collected, and the same Pd standard solution as that of Working Example 31 sufficient to give a concentration of 96 µg/L has been added to that water so as to prepare Pd colloid-containing base water 6.86. Radical scavenging activity measurement data for test water (AOW) obtained in conformity with the same testing procedures as those of

25 Working Example 61 when pre-electrolysis catalyst-added one-pass electrolyzed water is used as the test water (AOW), which is the Pd colloid-containing base water 6.86 prepared in the above-mentioned manner that has been subjected to electrolysis processing using a continuous flow method under the same conditions as those of Reference Example 23, is given as Working Example 62.

30 (Working Example 63)

1 liter of the same base water 6.86 as that of Reference Example 22 has been collected, and the same Pd standard solution as that of Working Example 31 sufficient to give a concentration of 192 µg/L has been added to that water so as to prepare Pd colloid-containing base water 6.86. Radical scavenging activity measurement data for

test water (AOW) obtained in conformity with the same testing procedures as those of Working Example 61 when pre-electrolysis catalyst-added one-pass electrolyzed water is used as the test water (AOW), which is the Pd colloid-containing base water 6.86 prepared in the above-mentioned manner that has been subjected to electrolysis

5   processing using a continuous flow method under the same conditions as those of Reference Example 23, is given as Working Example 63.

(Working Example 64)

1 liter of the same base water 6.86 as that of Reference Example 22 has been collected, and the same Pd standard solution as that of Working Example 31 sufficient

10   to give a concentration of 384 μg/L has been added to that water so as to prepare Pd colloid-containing base water 6.86. Radical scavenging activity measurement data for test water (AOW) obtained in conformity with the same testing procedures as those of Working Example 61 when pre-electrolysis catalyst-added one-pass electrolyzed water is used as the test water (AOW), which is the Pd colloid-containing base water 6.86

15   prepared in the above-mentioned manner that has been subjected to electrolysis processing using a continuous flow method under the same conditions as those of Reference Example 23, is given as Working Example 64.

(Working Example 65)

1 liter of the same base water 6.86 as that of Reference Example 22 has been

20   collected, and the same Pd standard solution as that of Working Example 31 sufficient to give a concentration of 768 μg/L has been added to that water so as to prepare Pd colloid-containing base water 6.86. Radical scavenging activity measurement data for test water (AOW) obtained in conformity with the same testing procedures as those of Working Example 61 when pre-electrolysis catalyst-added one-pass electrolyzed water

25   is used as the test water (AOW), which is the Pd colloid-containing base water 6.86 prepared in the above-mentioned manner that has been subjected to electrolysis processing using a continuous flow method under the same conditions as those of Reference Example 23, is given as Working Example 65.

(Working Example 66)

30   1 liter of the same base water 6.86 as that of Reference Example 22 has been collected, and the same Pt standard solution as that of Working Example 26 sufficient to give a concentration of 48 μg/L has been added to that water so as to prepare Pt colloid-containing base water 6.86. Radical scavenging activity measurement data for test water (AOW) obtained in conformity with the same testing procedures as those of

Reference Example 22 when pre-electrolysis catalyst-added circulating electrolyzed water is used as the test water (AOW), which is the Pt colloid-containing base water 6.86 prepared in this manner that has been subjected to electrolysis processing using a continuous flow circulating method (volume of circulatory water: 0.8 liters) for three

5   minutes under conditions of a 1.5 L/min flow and 5A constant current, is given as Working Example 66.

(Working Example 67)

    1 liter of the same base water 6.86 as that of Reference Example 22 has been collected, and the same Pt standard solution as that of Working Example 26 sufficient to

10   give a concentration of 96 µg/L has been added to that water so as to prepare Pt colloid-containing base water 6.86. Radical scavenging activity measurement data for test water (AOW) obtained in conformity with the same testing procedures as those of Working Example 66 when pre-electrolysis catalyst-added circulating electrolyzed water is used as the test water (AOW), which is the Pt colloid-containing base water

15   6.86 prepared in the above-mentioned manner that has been subjected to electrolysis processing using a continuous flow circulating method (volume of circulatory water: 0.8 liters) for three minutes under the same conditions as that of Working Example 66, is given as Working Example 67.

(Working Example 68)

20   1 liter of the same base water 6.86 as that of Reference Example 22 has been collected, and the same Pt standard solution as that of Working Example 26 sufficient to give a concentration of 192 µg/L has been added to that water so as to prepare Pt colloid-containing base water 6.86. Radical scavenging activity measurement data for test water (AOW) obtained in conformity with the same testing procedures as those of

25   Working Example 66 when pre-electrolysis catalyst-added circulating electrolyzed water is used as the test water (AOW), which is the Pt colloid-containing base water 6.86 prepared in the above-mentioned manner that has been subjected to electrolysis processing using a continuous flow circulating method (volume of circulatory water: 0.8 liters) for three minutes under the same conditions as that of Working Example 66, is

30   given as Working Example 68.

(Working Example 69)

    1 liter of the same base water 6.86 as that of Reference Example 22 has been collected, and the same Pt standard solution as that of Working Example 26 sufficient to give a concentration of 384 µg/L has been added to that water so as to prepare Pt

colloid-containing base water 6.86. Radical scavenging activity measurement data for test water (AOW) obtained in conformity with the same testing procedures as those of Working Example 66 when pre-electrolysis catalyst-added circulating electrolyzed water is used as the test water (AOW), which is the Pt colloid-containing base water

5   6.86 prepared in the above-mentioned manner that has been subjected to electrolysis processing using a continuous flow circulating method (volume of circulatory water: 0.8 liters) for three minutes under the same conditions as that of Working Example 66, is given as Working Example 69.

(Working Example 70)

10   1 liter of the same base water 6.86 as that of Reference Example 22 has been collected, and the same Pt standard solution as that of Working Example 26 sufficient to give a concentration of 768 µg/L has been added to that water so as to prepare Pt colloid-containing base water 6.86. Radical scavenging activity measurement data for test water (AOW) obtained in conformity with the same testing procedures as those of

15   Working Example 66 when pre-electrolysis catalyst-added circulating electrolyzed water is used as the test water (AOW), which is the Pt colloid-containing base water 6.86 prepared in the above-mentioned manner that has been subjected to electrolysis processing using a continuous flow circulating method (volume of circulatory water: 0.8 liters) for three minutes under the same conditions as that of Working Example 66, is

20   given as Working Example 70.

(Working Example 71)

1 liter of the same base water 6.86 as that of Reference Example 22 has been collected, and the same Pd standard solution as that of Working Example 31 sufficient to give a concentration of 48 µg/L has been added to that water so as to prepare Pd

25   colloid-containing base water 6.86. Radical scavenging activity measurement data for test water (AOW) obtained in conformity with the same testing procedures as those of Reference Example 22 when pre-electrolysis catalyst-added circulating electrolyzed water is used as the test water (AOW), which is the Pd colloid-containing base water 6.86 prepared in the above-mentioned manner that has been subjected to electrolysis

30   processing using a continuous flow circulating method (volume of circulatory water: 0.8 liters) for three minutes under conditions of a 1.5 L/min flow and 5A constant current, is given as Working Example 71.

(Working Example 72)

1 liter of the same base water 6.86 as that of Reference Example 22 has been

collected, and the same Pd standard solution as that of Working Example 31 sufficient to give a concentration of 96 μg/L has been added to that water so as to prepare Pd colloid-containing base water 6.86. Radical scavenging activity measurement data for test water (AOW) obtained in conformity with the same testing procedures as those of

5   Working Example 71 when pre-electrolysis catalyst-added circulating electrolyzed water is used as the test water (AOW), which is the Pd colloid-containing base water 6.86 prepared in the above-mentioned manner that has been subjected to electrolysis processing using a continuous flow circulating method (volume of circulatory water: 0.8 liters) for three minutes under the same conditions as those of Working Example 66, is

10   given as Working Example 72.

(Working Example 73)

1 liter of the same base water 6.86 as that of Reference Example 22 has been collected, and the same Pd standard solution as that of Working Example 31 sufficient to give a concentration of 192 μg/L has been added to that water so as to prepare Pd

15   colloid-containing base water 6.86. Radical scavenging activity measurement data for test water (AOW) obtained in conformity with the same testing procedures as those of Working Example 71 when pre-electrolysis catalyst-added circulating electrolyzed water is used as the test water (AOW), which is the Pd colloid-containing base water 6.86 prepared in the above-mentioned manner that has been subjected to electrolysis

20   processing using a continuous flow circulating method (volume of circulatory water: 0.8 liters) for three minutes under the same conditions as those of Working Example 66, is given as Working Example 73.

(Working Example 74)

1 liter of the same base water 6.86 as that of Reference Example 22 has been

25   collected, and the same Pd standard solution as that of Working Example 31 sufficient to give a concentration of 384 μg/L has been added to that water so as to prepare Pd colloid-containing base water 6.86. Radical scavenging activity measurement data for test water (AOW) obtained in conformity with the same testing procedures as those of Working Example 71 when pre-electrolysis catalyst-added circulating electrolyzed

30   water is used as the test water (AOW), which is the Pd colloid-containing base water 6.86 prepared in the above-mentioned manner that has been subjected to electrolysis processing using a continuous flow circulating method (volume of circulatory water: 0.8 liters) for three minutes under the same conditions as those of Working Example 66, is given as Working Example 74.

(Working Example 75)

1 liter of the same base water 6.86 as that of Reference Example 22 has been collected, and the same Pd standard solution as that of Working Example 31 sufficient to give a concentration of 768 µg/L has been added to that water so as to prepare Pd

5 colloid-containing base water 6.86. Radical scavenging activity measurement data for test water (AOW) obtained in conformity with the same testing procedures as those of Working Example 71 when pre-electrolysis catalyst-added circulating electrolyzed water is used as the test water (AOW), which is the Pd colloid-containing base water 6.86 prepared in the above-mentioned manner that has been subjected to electrolysis

10 processing using a continuous flow circulating method (volume of circulatory water: 0.8 liters) for three minutes under the same conditions as those of Working Example 66, is given as Working Example 75.

(Working Example 76)

Radical scavenging activity measurement data obtained in conformity with

15 testing procedures altered the same as with Reference Example 21 when AOW is used as test water, which is obtained by adding to distilled water the same Pt standard solution as that of Working Example 26 sufficient to give a Pt colloid concentration of 384 µg/L, and then subjecting it to hydrogen gas replacement, is given as Working Example 76.

20 (Working Example 77)

Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Working Example 76 when AOW is used as test water, which is obtained by adding to distilled water the same Pd standard solution as that of Working Example 31 sufficient to give a Pd colloid concentration of 384 µg/L, and

25 then that subjecting it to hydrogen gas replacement, is given as Working Example 77.

(Reference Example 24)

Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Reference Example 19 when an AsA solution is used as test water, which is obtained by adding to distilled water ascorbic acid (AsA)

30 sufficient to give a concentration of 35.5 µM, is given as Reference Example 24.

(Reference Example 25)

Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Reference Example 19 when AsA solution is used as test water, which is obtained by adding to distilled water ascorbic acid (AsA) sufficient

to give a concentration of 71 µM, is given as Reference Example 25.

(Reference Example 26)

Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Reference Example 19 when AsA solution is used as

5   test water, which is obtained by adding to distilled water ascorbic acid (AsA) sufficient to give a concentration of 142 µM, is given as Reference Example 26.

(Reference Example 27)

Radical scavenging activity measurement data obtained in conformity with the same testing procedures as those of Reference Example 19 when AsA solution is used as

10   test water, which is obtained by adding to distilled water ascorbic acid (AsA) sufficient to give a concentration of 284 µM, is given as Reference Example 27.

(C) Examination of working examples

FIG. 26, which compares Reference Examples 19, 20, and Working Examples 26 through 30, shows characteristics of the radical scavenging activity changing over

15   time when Pt colloid catalyst-containing hydrogen-dissolved water (AOW) is expressed where Pt colloid (particle size distribution: 2 through 4 nm) concentration is used as a main parameter. Here, Reference Examples 19 and 20 are both used as references in order to grasp the profiles of the radical scavenging activity characteristics when oxygen is removed from test water (fluid) occupying 1/3 of the tester cell capacity of 3

20   mL. It should be noted that it has been confirmed through an experiment not shown in the drawings that even when nitrogen gas-replaced distilled water is employed as test water, instead of the hydrogen gas-replaced distilled water of Reference Example 20, exactly the same characteristics of the radical scavenging activity changing over time can be obtained. This means that the type of gas used as a reference does not affect the

25   characteristics of the radical scavenging activity changing over time as long as the same amount of oxygen is removed (hereafter, the same). Here, according to this drawing, when compared to the radical scavenging activity characteristics of Reference Examples 19 and 20 (hereafter, the characteristics of reference examples are abbreviated as 'reference characteristics'), the radical scavenging activity characteristics of AOWs of

30   Working Examples 26 through 30 (hereafter, characteristics of working examples are abbreviated as 'subject characteristics') are significantly lower than the reference characteristics for each concentration after approximately 680 seconds from the start of measurement of change over time (hereafter, abbreviated as 'start of measurement') of the light absorbance (A480) using a spectrophotometer. In other words, it can be

understood that AOWs of Working Examples 26 through 30 begin to express the favorable radical scavenging activity in a wide concentration range after a certain time period has passed. In addition, analyzing the subject characteristics of Working Examples 26 through 30 in detail shows that the higher the Pt colloid concentration, the

5 shorter the time required for radical scavenging. In other words, the radical scavenging activity expressed in AOW becomes higher depending on the Pt colloid concentration. It should be noted that when Pt colloid is employed as a precious metal catalyst, there are time ranges where the subject characteristics of Working Examples 26 through 30 are significantly higher than the reference characteristics for each

10 concentration. The reason thereof will be described in the following section 'non-reactivity against oxidant, catalytic activity, and hydrogen-occluding capability of palladium (Pd) colloid', therefore it is no longer referred to at this time (hereafter, the same).

FIG. 27, which compares Reference Examples 19, 20, and Working Examples

15 31 through 35, shows characteristics of the radical scavenging activity changing over time when Pd colloid catalyst-containing hydrogen-dissolved water (AOW) is expressed where Pd colloid concentration is used as a main parameter. According to this drawing, when comparing the subject characteristics of Working Examples 31 through 35 to the reference characteristics of Reference Examples 19 and 20, in almost all time ranges

20 from the start of measurement, the subject characteristics of AOW with a low concentration (Working Examples 31 through 33) are almost equivalent to the reference characteristics, but the subject characteristics of AOW with a high concentration (Working Examples 34 and 35) are all significantly lower than the reference characteristics. In other words, it can be understood that AOWs of Working Examples

25 31 through 35 begin to express favorable radical scavenging activity with high concentrations (Working Examples 34 and 35). In addition, analyzing the subject characteristics of Working Examples 34 and 35 in detail shows that the higher the Pd colloid concentration, the shorter the time required for radical scavenging. In other words, the radical scavenging activity expressed in AOW becomes higher depending on

30 the Pd colloid concentration. In particular, with the subject characteristics of AOW with a high concentration (Working Examples 34 and 35), the light absorption level begins to show a significant decrease after approximately 440 seconds and approximately 230 seconds, respectively, from the start of measurement. The reason can be guessed that since the ($\cdot O_2^-$) existence concentration (existing amount) acts as a

switch for AOW with a high concentration (Working Examples 34 through 35), it begins to be aggressively scavenged. It should be noted that when Pd colloid is employed as a precious metal catalyst, the subject characteristics of Working Examples 31 through 35 are mostly lower than the reference characteristics for each concentration. The reason

5   thereof will be described in the following section 'non-reactivity against oxidant, catalytic activity, and hydrogen occlusion capability of palladium (Pd) colloid', and is therefore not described here (hereafter, the same).

FIG. 28, which compares Reference Examples 19, 20, and Working Examples 36 through 41, shows characteristics of the radical scavenging activity changing over

10   time when Pt colloid catalyst-containing hydrogen-dissolved water (AOW) is expressed where Pt colloid (particle size distribution: 2 through 4 nm) concentration is used as a main parameter. According to this drawing, when compared to the reference characteristics of Reference Examples 19 and 20, the subject characteristics of Working Examples 36 through 41 are significantly lower than the reference characteristics for

15   each concentration after approximately 920 seconds from the start of measurement. In other words, it can be understood that AOWs of Working Examples 36 through 41 begin to express the favorable radical scavenging activity in a wide concentration range after a certain time period has passed. In addition, analyzing the subject characteristics of Working Examples 36 through 41 in detail shows that the higher the Pt colloid

20   concentration, the shorter the time required for radical scavenging. In other words, the radical scavenging activity expressed in AOW becomes higher depending on the Pt colloid concentration.

Here, in order to clarify the relationship between the particle size parameters (particle size distribution: 2 to 4 nm/ particle size distribution: 1 to 2 nm) of Pt colloid

25   and the radical scavenging activity, FIG. 26 where Pt colloid concentration with a particle size distribution of 2 to 4 nm is used as a main parameter is compared to FIG. 28 where Pt colloid concentration with a particle size distribution of 1 to 2 nm is used as a main parameter. In order to avoid influence from the concentration parameter, for example, Working Examples 27 and 37 using the same Pt concentration parameter (96

30   μg/L) are compared. Working Example 27 shows that the subject characteristic such as the light absorption level reaches a peak value after approximately 320 seconds from the start of measurement, and then gradually decreasing, and after approximately 440 seconds the light absorption level is once controlled to almost 0. Meanwhile, Working Example 37 shows that the subject characteristic such as the light absorption level

reaches a peak value after approximately 760 seconds from the start of measurement, and then gradually decreasing, but the light absorption level is not controlled to almost 0 within the measurement time range. Next, Working Examples 29 and 40 using the same Pt concentration parameter (384 μg/L) are compared. Working Example 29 shows that the subject characteristic is controlled to almost 0 after approximately 170 seconds from the start of measurement, and that decreasing continues thereafter. Meanwhile, Working Example 40 shows that the subject characteristic such as the light absorption level reaches a peak value after approximately 260 seconds from the start of measurement, and then rapidly decreasing, and the light absorption level is controlled to almost 0 after approximately 340 seconds. Comprehensively viewing the above-mentioned findings, a Pt colloid catalyst having a particle size distribution of 2 to 4 nm is more preferable than that having a particle size distribution of 1 to 2 nm as the Pt colloid catalyst to be used for the antioxidant-functioning water according to the present invention since having a particle size distribution of 2 to 4 nm can provide a better expression of the radical scavenging activity (which emanates from the fact that the time for control of the light absorption level to almost 0 is shorter.)

FIG. 29, which compares Reference Examples 19, 20, and Working Examples 42 through 45, shows characteristics of the radical scavenging activity changing over time when mixed (Pt + Pd) colloid catalyst-containing hydrogen-dissolved water (AOW) is expressed where mixed (Pt + Pd) colloid (particle size distribution: 2 through 4 nm, Pt to Pd mixture mole ratio: 1 to 2) concentration is used as a main parameter. According to this drawing, when compared to the reference characteristics of Reference Examples 19 and 20, the subject characteristics of Working Examples 42 through 45 are significantly lower than the reference characteristics for each concentration after approximately 900 seconds from the start of measurement. In other words, it can be understood that AOWs of Working Examples 42 through 45 begin to express the favorable radical scavenging activity in a wide concentration range after a certain time period has passed. In addition, analyzing the subject characteristics of Working Examples 42 through 45 in detail shows that the higher the mixed (Pt + Pd) colloid concentration, the shorter the time required for radical scavenging. In other words, the radical scavenging activity expressed in AOW becomes higher depending on the mixed (Pt + Pd) colloid concentration.

FIG. 30, which compares Reference Examples 19, 20, and Working Examples 46 through 50, shows characteristics of the radical scavenging activity changing over

time when mixed (Pt + Pd) colloid catalyst-containing hydrogen-dissolved water (AOW) is expressed where mixed (Pt + Pd) colloid (particle size distribution: 2 through 4 nm) concentration is used as a main parameter and Pt to Pd mixture mole ratio is used as a sub parameter. According to this drawing, when compared to the reference

5 characteristics of Reference Examples 19 and 20, the subject characteristics of Working Examples 46 through 50 are significantly lower than the reference characteristics for each concentration after approximately 520 seconds from the start of measurement. In other words, it can be understood that AOWs of Working Examples 46 through 50 begin to express the favorable radical scavenging activity in a wide concentration range after

10 a certain time period has passed. In addition, analyzing the subject characteristics of Working Examples 46 through 50 in detail shows that the higher the mixed (Pt + Pd) colloid concentration, the shorter the time required for radical scavenging. In other words, the radical scavenging activity expressed in AOW becomes higher depending on the mixed (Pt + Pd) colloid concentration.

15 FIG. 31, which compares Reference Example 21, and Working Examples 51 through 55, shows characteristics of the radical scavenging activity changing over time when mixed (Pt + Pd) colloid catalyst-containing hydrogen-dissolved water (AOW) is expressed where mixed (Pt + Pd) colloid (particle size distribution: 2 through 4 nm) concentration is used as a main parameter and Pt to Pd mixture mole ratio is used as a

20 sub parameter. It should be noted that in Reference Example 21 and Working Examples 46 through 50, the ($\cdot O_2^-$) generation system is removed. According to this drawing, when compared to the reference characteristics of Reference Example 21, an ($\cdot O_2^-$) generating tendency is found in the subject characteristics of Working Examples 51 through 55 even though the ($\cdot O_2^-$) generation system has been removed. Since this

25 point is described in the following description of FIG. 37, it is no longer referred to at this time. This ($\cdot O_2^-$) generating tendency depends on the mixed (Pt + Pd) colloid concentration, that is, the higher the concentration becomes, the more this ($\cdot O_2^-$) generating tendency is controlled. In other words, the radical scavenging activity expressed in AOW becomes higher depending on the mixed (Pt + Pd) colloid

30 concentration. In addition, comprehensively taking into consideration the phenomena in relation to FIG. 37 to be described later, it can be concluded that the higher the mixed mole ratio of Pd colloid becomes, the more the ($\cdot O_2^-$) generating tendency is controlled.

FIG. 32, which compares Reference Examples 22, 23, and Working Examples 56 through 60, shows characteristics of the radical scavenging activity changing over

time when pre-electrolysis Pd colloid catalyst-added one-pass electrolyzed water (AOW) is expressed where Pt colloid (particle size distribution: 2 to 4 nm) concentration is used as a main parameter. According to this drawing, the subject characteristics of AOW with a low concentration of the Working Examples 56 through 60 (Working Examples 66

5   and 57) are almost equivalent to the reference characteristics, but the subject characteristics of AOW with a high concentration (Working Examples 58 through 70) are significantly lower than the reference characteristics for each concentration after approximately 740 seconds from the start of measurement. In other words, it can be understood that AOWs of Working Examples 58 through 60 begin to express the

10   favorable radical scavenging activity in a wide concentration range after a certain time period has passed. In addition, analyzing the subject characteristics of Working Examples 58 through 60 in detail shows that the higher the Pt colloid concentration, the shorter the time required for radical scavenging. In other words, the radical scavenging activity expressed in AOW becomes higher depending on the Pt colloid

15   concentration.

FIG. 33, which compares Reference Examples 22, 23, and Working Examples 61 through 65, shows characteristics of the radical scavenging activity changing over time when pre-electrolysis Pd colloid catalyst-added one-pass electrolyzed water is expressed where Pd colloid (particle size distribution: 2 through 4 nm) is used as a main

20   parameter. According to this drawing, the subject characteristics of AOW with a low concentration of the Working Examples 61 through 65 (Working Examples 61 and 62) are almost equivalent to the reference characteristics, but the subject characteristics of AOW with a high concentration (Working Examples 63 through 65) are significantly lower than the reference characteristics for each concentration after approximately 320

25   seconds from the start of measurement. In other words, it can be understood that AOWs of Working Examples 63 through 65 begin to express the favorable radical scavenging activity in a wide concentration range after a certain time period has passed. In addition, analyzing the subject characteristics of Working Examples 63 through 65 in detail shows that the higher the Pd colloid concentration, the shorter the time required

30   for radical scavenging. In other words, the radical scavenging activity expressed in AOW becomes higher depending on the Pd colloid concentration. In particular, in the case of the subject characteristics of AOW with the highest concentration (Working Example 65), the light absorption level begins to show a significant decrease after approximately 830 seconds from the start of measurement. The reason can be guessed

that since the ($\cdot O_2^-$) existence concentration (existing amount) acts as a switch for AOW with a high concentration (Working Example 65), it begins to be aggressively scavenged.

FIG. 34, which compares Reference Examples 22, 23, and Working Examples 66 through 70, shows characteristics of the radical scavenging activity changing over time when pre-electrolysis Pd colloid catalyst-added circulating electrolyzed water (AOW) is expressed where Pt colloid (particle size distribution: 2 through 4 nm) concentration is used as a main parameter. According to this drawing, the subject characteristics of AOW with a low concentration of the Working Examples 66 through 70 (Working Examples 66 and 67) are almost equivalent to the reference characteristics, but the subject characteristics of AOW with a high concentration (Working Examples 68 through 70) are significantly lower than the reference characteristics for each concentration after approximately 700 seconds from the start of measurement. In other words, it can be understood that AOWs of Working Examples 68 through 70 begin to express the favorable radical scavenging activity in a wide concentration range after a certain time period has passed. In addition, analyzing the subject characteristics of Working Examples 68 through 70 in detail shows that the higher Pt colloid concentration, the shorter the time required for radical scavenging. In other words, the radical scavenging activity expressed in AOW becomes higher depending on the Pt colloid concentration.

FIG. 35, which compares Reference Examples 22 and 23 and Working Examples 71 through 75, shows characteristics of the radical scavenging activity changing over time when pre-electrolysis Pd colloid catalyst-added circulating electrolyzed water (AOW) is expressed where Pd colloid (particle size distribution is 2 through 4 nm) concentration is used as a main parameter. According to this drawing, while the subject characteristics of AOW with a low concentration of the Working Examples 71 through 75 (Working Examples 71 and 72) show a slight downward tendency after approximately 950 seconds from the start of measurement, the subject characteristics of AOW with a high concentration (Working Examples 73 through 75) are significantly lower than the reference characteristics for each concentration after approximately 650 seconds from the start of measurement. In other words, it can be understood that AOWs of Working Examples 71 through 75 begin to express the favorable radical scavenging activity in a wide concentration range after a certain time period has passed. In addition, analyzing the subject characteristics of these Working Examples 71 through 75 in detail shows that the higher the Pd colloid concentration,

the shorter the time required for radical scavenging. In other words, the radical scavenging activity expressed in AOW becomes higher depending on the Pd colloid concentration. In particular, with the subject characteristics of AOW with high concentration (Working Examples 73 through 75), the light absorption level begins to

5 show a significant decrease after approximately 650 seconds, approximately 420 seconds, and approximately 230 seconds, respectively, from the start of measurement. The reason can be guessed that since the ($\cdot O_2^-$) existence concentration (existing amount) acts as a switch for AOW with a high concentration (Working Examples 73 through 75), it begins to be aggressively scavenged.

10 Here, in order to clarify the relationship between electrolysis condition parameters for a Pt colloid catalyst (one-pass electrolysis/circulating electrolysis) and the radical scavenging activity, FIG. 32 where pre-electrolysis Pd colloid catalyst-added one-pass electrolyzed water is employed as test water is compared to FIG. 34 where pre-electrolysis Pd colloid catalyst-added circulating electrolyzed water is employed as

15 test water. In order to avoid influence from the concentration parameter, for example, Working Examples 58 and 68 using the same Pt concentration parameter (192 µg/L) are compared. Working Example 58 shows that the subject characteristic such as the light absorption level reaches its peak after approximately 680 seconds from the start of measurement, then gradually decreasing, and after approximately 880 seconds the light

20 absorption level is controlled to almost 0. Meanwhile, Working Example 68 shows that the subject characteristic such as the light absorption level reaches its peak after approximately 620 seconds from the start of measurement, then gradually decreasing, and after approximately 830 seconds the light absorption level is controlled to almost 0. Next, Working Examples 59 and 69 using the same Pt concentration parameter (384

25 µg/L) are compared. Working Example 59 shows that the subject characteristic such as the light absorption level reaches its peak after approximately 530 seconds from the start of measurement, then gradually decreasing, and after approximately 660 seconds the light absorption level is controlled to almost 0. Meanwhile, Working Example 69 shows that the subject characteristic such as the light absorption level reaches its peak

30 after approximately 400 seconds from the start of measurement, then gradually decreasing, and after approximately 500 seconds the light absorption level is controlled to almost 0. Synthetically viewing the above-mentioned findings, the circulating electrolysis is more preferable than the one-pass electrolysis as the electrolysis condition (only for electrolysis after the Pd colloid catalyst has been added) for

generating antioxidant-functioning water according to the present invention since that circulating electrolysis can provide a better expression of the radical scavenging activity (which emanates from the fact that the time for control of the light absorption level to almost 0 is shorter.)

5   On the other hand, in order to clarify the relationship between electrolysis condition parameters for a Pd colloid catalyst (one-pass electrolysis/ circulating electrolysis) and the radical scavenging activity, FIG. 33 where pre-electrolysis Pd colloid catalyst-added one-pass electrolyzed water is employed as test water is compared to FIG. 35 where pre-electrolysis Pd colloid catalyst-added circulating electrolyzed water is employed as test water.   In order to avoid influence from the concentration

10   parameter, for example, Working Examples 63 and 73 using the same Pd concentration parameter (192 µg/L) are compared.   In Working Example 63, while the subject characteristics thereof are lower than the reference characteristics of Reference Examples 22 and 23 in all time ranges from the start of measurement, those subject

15   characteristics show a gradual upward tendency.   Meanwhile, the subject characteristics of Working Example 73 show a gradual upward tendency until reaching approximately 650 seconds (peak of light absorption level) from the start of measurement.   In turn, those subject characteristics then show a gradual downward tendency, and the light absorption level is controlled to almost 0 after approximately

20   860 seconds.   Next, Working Examples 65 and 75 using the same Pd concentration parameter (768 µg/L) are compared.   In Working Example 65, the subject characteristics show a gradual upward tendency until reaching approximately 680 to 800 seconds (peak of light absorption level) from the start of measurement.   In turn, those subject characteristics then show a gradual downward tendency.   Meanwhile,

25   Working Example 75 shows that the subject characteristic such as the light absorption level reaches its peak upon start of measurement, then gradually decreasing, and after approximately 320 seconds the light absorption level is controlled to almost 0. Synthetically viewing the above-mentioned findings, the circulating electrolysis is more preferable than the one-pass electrolysis as the electrolysis condition (only for

30   electrolysis after the Pd colloid catalyst has been added) for generating antioxidant-functioning water according to the present invention since that circulating electrolysis can provide a better expression of the radical scavenging activity (which emanates from the fact that the time for control of the light absorption level to almost 0 is shorter.)

FIG. 36, which compares Reference Examples 19, 20, 24 through 27, shows characteristics of the radical scavenging activity changing over time when AsA solution is expressed where AsA solution concentration is used as a main parameter. According to this drawing, the radical scavenging activity characteristic of the AsA solution of

5   Reference Examples 24 through 27 is lower than the characteristic of Reference Examples 19 and 20 for each concentration. In other words, a well-known fact can be confirmed that the radical scavenging activity is expressed in AsA solutions of Reference Examples 24 through 27 in a wide concentration range. In addition, another well-known fact can also be confirmed that the radical scavenging activity expressed in

10   the AsA solutions of Reference Examples 24 through 27 becomes higher depending on the concentration. It should be noted that when comparing the radical scavenging activity expressed in the above-mentioned AsA solution to the radical scavenging activity expressed in antioxidant-functioning water according to the present invention, it can be known that, for example, the radical scavenging activity expressed in the

15   antioxidant-functioning water of Working Example 75 exceeds by far that expressed in the AsA solution of Reference Example 24. Therefore, the radical scavenging activity, which is comparable to the AsA solution of Reference Examples 25 through 27, is expressed in that antioxidant-functioning water.

Non-reactivity against oxidant, catalytic activity, and hydrogen-occluding capability of

20   palladium (Pd) colloid

Existence of oxygen becomes a large barrier when the antioxidation method, antioxidant-functioning water, and usage thereof according to the present invention are attempted to be implemented in an oxygen-dissolved solution system such as a living organism. The living organisms are especially abundant in oxygen since oxygen is

25   used to procure energy by oxidizing nutrients and perform various oxygen-added reactions essential for the living organisms. The true nature of the problem is decay in radical scavenging activity emanating from the fact that oxygen will consume the hydrogen dissolved in the antioxidant-functioning water by the assistance of a precious metal colloid catalyst, in other words, hydrogen and oxygen react to each other through

30   a precious metal colloid catalyst, reverting to normal water, as well as a scavenging subject ($\cdot O_2^-$) is adversely generated since the oxygen itself is reduced of one electron by the hydrogen, which is activated through a precious metal colloid catalyst. There is a tendency that the higher the catalytic activity becomes, the more the phenomenon according to this preposition increases. In short, since catalytic activity and radical

scavenging activity have a trade-off relationship, the higher the catalytic activity becomes, the more the radical scavenging activity decays. Accordingly, it can be said that this is a deep-seated problem that cannot be easily solved.

     As eager research progressed in order to solve such substantial problems, the

5   inventors found that in the precious metal colloid catalysts, palladium (Pd) colloid especially shows non-reactivity against oxidant in comparison with platinum (Pt) colloid. As further research progressed based on this discovery, the inventors clarified that the important factors to be taken into account when searching for precious metal colloid catalysts available for the present invention are: non-reactivity

10   against oxidant, catalytic activity, and hydrogen occlusion capability. Taking these three factors into consideration, the inventors have finally found that the favorable precious metal colloid catalyst is Pd colloid from the standpoint of overall capacity, thereby completing the invention.

    To begin with, grounds for the conclusion that Pd colloid shows non-reactivity

15   against oxidant in comparison with Pt colloid are described below.

    FIG. 37, which compares Reference Example 21, Working Examples 76, and 77, shows characteristics of the radical scavenging activity changing over time when catalyst-containing hydrogen-dissolved water (AOW) is expressed where difference in a type of a precious metal catalyst (concentration is fixed) is used as a main parameter.

20   It should be noted that in Reference Example 21 and Working Examples 76 through 77, the ($\cdot O_2^-$) generation system is removed. This drawing shows that although the ($\cdot O_2^-$) generation system is removed, the subject characteristic such as the light absorbance level for Working Example 76 reaches a peak value of approximately 0.046 (when approximately 160 seconds have passed) between approximately 140 to 200 seconds

25   from the start of measurement. It also shows that the light absorbance level gradually increases after approximately 860 seconds from the start of measurement. On the other hand, the subject characteristic of Working Example 77 shows almost the same tendency as the reference characteristic of Reference Example 21, but the light absorbance does not rise. These may be thought that in the subject characteristic of

30   Working Example 76, ($\cdot O_2^-$) has been actively generated until approximately 160 seconds from the start of measurement, and the ($\cdot O_2^-$) generated in the above-mentioned manner is scavenged by the radical scavenging expressed in antioxidant-functioning water according to the present invention. In addition, the reason why the light absorbance level turns to gradually increase after approximately 860 seconds from the

start of measurement may be because generation of ($\cdot O_2^-$) cannot be controlled and scavenged as a result of the radical scavenging activity of the antioxidant-functioning water according to the present invention having decayed or having been sapped.

Next, a theory regarding the working-action mechanism of the Pt colloid catalyst and the Pd colloid catalyst in a solution system where hydrogen and oxygen coexist (a solution system where oxygen is dissolved in hydrogen-dissolved water of the present invention) is explained.

FIG. 38 shows the working-action mechanism of the Pt colloid catalyst in the hydrogen-oxygen coexistent solution system.

As shown in this drawing, the Pt colloid catalyst absorbs hydrogen and oxygen, which are dissolved in the system, and passes to the oxygen one electron released from the activated hydrogen ($\cdot H$) (one-electron reduction of oxygen.) At this time, the activated hydrogen ($\cdot H$) loses one electron, and is released to the system as an H^+ ion (hereafter, repetitive description hereof is omitted.) That is, since the oxygen itself is reduced of one electron by the activated hydrogen through the Pt colloid catalyst, ($\cdot O_2^-$) is adversely generated although it is a primary scavenging subject. Thereafter, or at the same time, the Pt colloid catalyst absorbs ($\cdot O_2^-$) and hydrogen dissolved in the system, and passes to the ($\cdot O_2^-$) one electron released from the activated hydrogen (one-electron reduction of ($\cdot O_2^-$) or two-electron reduction of oxygen.) In short, when ($\cdot O_2^-$) itself is reduced of one electron by the activated hydrogen through Pt colloid catalyst, ($\cdot O_2^{2-}$) is generated. An ionic bond is formed between the ($\cdot O_2^{2-}$) generated in this manner and two H^+ ions existing in the system, generating hydrogen peroxide (H_2O_2). Hereafter, or at the same time, Pt colloid catalyst absorbs hydrogen and hydrogen peroxide (H_2O_2), which are dissolved in the system, and passes to the (H_2O_2) one electron released from the activated hydrogen (one-electron reduction of (H_2O_2) or three-electron reduction of oxygen). In short, when the (H_2O_2) itself is reduced of one electron by the activated hydrogen through Pt colloid catalyst, ($\cdot OH$) is generated. Thereafter, or at the same time, Pt colloid catalyst absorbs hydrogen and ($\cdot OH$), which are dissolved in the system, and passes to the ($\cdot OH$) one electron released from the activated hydrogen (one-electron reduction of ($\cdot OH$) or four-electron reduction of oxygen). In short, when ($\cdot OH$) itself is reduced of one electron by the activated hydrogen through Pt colloid catalyst, an OH^- ion is then generated. An ionic bond is formed between the OH^- generated in this manner and an H^+ ion, generating water (H_2O), and ending a series of reactions. The working-action mechanism of the Pt

colloid catalyst in the solution system where hydrogen and oxygen coexist has been explained.

Meanwhile, the working-action mechanism of the Pd colloid catalyst is described forthwith.

FIG. 39 shows the working-action mechanism of Pd colloid catalyst in a hydrogen-oxygen coexistent solution system. It should be noted that since the greatest difference in the description of the working-action mechanism of the Pd colloid catalyst from the above-mentioned working-action mechanism of the Pt colloid catalyst is something originating from non-reactivity against oxidant, this is mainly described, and other repetitive descriptions are omitted.

As shown in this drawing, while the Pd colloid catalyst absorbs hydrogen dissolved in the system, it does not absorb oxygen actively, or it tends to be difficult to pass one electron released from the activated hydrogen (·H) to oxygen even if passive absorption (due to collision of oxygen to Pd colloid catalyst) occurs (no oxygen molecules reduced). Accordingly, hardly any (·O_2^-), which is a primary scavenging subject, is generated. The following working-action mechanism is the same as that of the Pt colloid catalyst, that is, (·O_2^-), hydrogen peroxide (H_2O_2), or (·OH) existing in the system is reduced by the Pd colloid catalyst and hydrogen, which is dissolved in the system, collaboratively; wherein water (H_2O) is ultimately generated, ending a series of reactions.

Here, to give the characteristics of palladium (Pd), which is favorable as the precious metal colloid catalyst according to the present invention, palladium has atomic number 46, an atomic mass of 106.42, and is a transition metal atom, which was discovered by Wollaston in 1803. This has been named after the asteroid Pallas (Athens in Greek mythology) which was discovered the previous year. It is a precious atom with only 24,000 tons existing on the earth. Palladium has excellent hydrogen-capturing capabilities, where it can occlude 740 to 935 times its own volume. It is frequently used as a hydrogenising catalyst and for purifying hydrogen. Palladium is most frequently used as a catalyst. It has been used as a hydrogenising catalyst, as well as a palladium complex has been used as a catalyst which is used to generate acetaldehyde from ethylene. Palladium is also used as a metal for dental treatment and decorative trim.

To think about a reactive subject to the antioxidant-functioning water according to the present invention, for example, the reactive subject can be roughly

classified into a free radical with unpaired electrons and strong oxidizing power, and an oxidizing material without unpaired electrons but oxidizing power. Of these, in the former case, radical scavenging activity may be expressed due to molecular hydrogen with a strong reducing power, which emanates from the antioxidant-functioning water according to the present invention, corresponding to the radical (i.e., a molecular hydrogen electron is given to the radical.) Meanwhile, in the latter case, reduction activity may be expressed selectively due to molecular hydrogen with a strong reducing power, which emanates from the antioxidant-functioning water according to the present invention, selectively corresponding to the oxidizing material according to the target (i.e., an electron of the molecular hydrogen may be given according to the target.) Here, selective expression of the reduction activity may mean that the reduction activity is selectively expressed depending on the condition of a degree allowing easy flow of frontier electrons, which occupy the highest occupied orbital on the molecular hydrogen side, to the lowest altitude orbit on the oxidizing material side according to the compatibility between the molecular hydrogen and oxidizing material, that is, the frontier electron theory. Giving a specific example just for reference, in test water where vitamin B2 or an oxidizing material is dissolved in Pd colloid catalyst-containing (concentration: 192 μg/L) hydrogen-dissolved water (AOW) according to the present invention, the reduction activity of vitamin B2 cannot be found. In this case, reduction activity can not be expressed because the molecular hydrogen with a strong reducing power emanating from the antioxidant-functioning water according to the present invention gave no electrons to the oxidizing material (vitamin B2). In short, it can be said that molecular hydrogen and oxidizing material (vitamin B2) are incompatible. On the other hand, in the test water where oxidized methylene blue or an oxidizing material is dissolved in the Pd colloid catalyst-containing (concentration: 192 μg/L) hydrogen-dissolved water according to the present invention, the reduction activity of methylene blue can be found. In this case, the reduction activity can be found since molecular hydrogen with a strong reducing power emanating from the antioxidant-functioning water according to the present invention gave electrons to the oxidizing material (oxidized methylene blue). In short, it can be said that molecular hydrogen and oxidizing material (oxidized methylene blue) are congenial.

In order to enhance the reactivity of the antioxidant-functioning water according to the present invention to the above-mentioned reaction subject, the fact that for example, the antioxidant-functioning water dissolves hydrogen with a certain

concentration higher than the saturated concentration (in terms of the effective value of dissolved hydrogen concentration found using a dissolved hydrogen concentration quantitative analysis method that uses oxidation/ reduction pigment) under atmospheric pressure, and/or that a considerable amount of hydrogen is occluded in the

5   precious metal catalyst itself contained in the hydrogen-dissolved water may be favorable.

Disclosure of additional working examples by DH quantitative analysis method using oxidation/reduction pigment

Additional working examples by a DH quantitative analysis method using the

10 above-mentioned oxidation/ reduction pigment are described below.

(Working Example 78)

Using the same pre-electrolysis catalyst-added circulating electrolyzed water as that of Working Example 73 as test water (AOW), 1 mL of one-fortieth strength Pt standard solution that has undergone the nitrogen gas replacement described above is

15 injected to 200 mL of this test water in a test water holding compartment using a syringe. This is then sufficiently stirred and mixed, and thereafter while visually observing the color change of the test water, a 10 g/L concentration (mole concentration by volume: 26773.8 µM) of methylene blue solution is injected a little bit at a time into the test water using a syringe. The total amount of methylene blue solution injected

20 until reaching the end point was 7.8 mL, and the measured dissolved hydrogen concentration DH found by substituting each value into Equation 7 was 2.09 (mg/L). Each physical property value of the test water according to this Working Example 78 is shown in Table 4, and the effective value of the dissolved hydrogen concentration DH is shown in FIG. 40.

25 (Working Example 79)

Using catalyst-free circulating electrolyzed water as test water, which is the same base water 6.86 as that of Reference Example 22 that has been subjected to electrolysis processing using a continuous flow circulating method (volume of circulatory water: 0.8 liters) for three minutes under the same electrolysis conditions as

30 those of Working Example 71, 1 mL of one-fortieth strength Pt standard solution that has undergone the nitrogen gas replacement described above is injected to 200 mL of this test water in a test water holding compartment using a syringe. This is then sufficiently stirred and mixed, and thereafter while visually observing the color change of the test water, a 10 g/L concentration (mole concentration by volume: 26773.8 µM) of

methylene blue solution is injected a little bit at a time into the test water using a syringe. The total amount of methylene blue solution injected until reaching the end point was 8.5 mL, and the measured dissolved hydrogen concentration DH found by substituting each value into Equation 7 was 2.28 (mg/L). Each physical property value

5 of the test water according to this Working Example 79 is shown in Table 4, and the effective value of the dissolved hydrogen concentration DH is shown in FIG. 40.

(Working Example 80)

Pd colloid-containing activated charcoal processed water, which is obtained by adding the same Pd standard solution as that of Working Example 31 sufficient to give

10 a concentration of 384 μg/L to activated charcoal processed water resulting from processing Fujisawa City tap water through an activated charcoal column, is prepared. Using pre-electrolysis catalyst-added circulating electrolyzed water as test water (AOW), which is Pd colloid-containing activated charcoal processed water prepared in this manner that has been subjected to electrolysis processing using a continuous flow

15 circulating method (volume of circulatory water: 0.8 liters) for three minutes under the same conditions as those of Working Example 71, 1 mL of one-fortieth strength Pt standard solution that has undergone the nitrogen gas replacement described above is injected to 200 mL of this test water in a test water holding compartment using a syringe. This is then sufficiently stirred and mixed, and thereafter while visually

20 observing the color change of the test water, a 10 g/L concentration (mole concentration by volume: 26773.8 μM) of methylene blue solution is injected a little bit at a time into the test water using a syringe. The total amount of methylene blue solution injected until reaching the end point was 9.7 mL, and the measured dissolved hydrogen concentration DH found by substituting each value into Equation 7 was 2.60 (mg/L).

25 Each physical property value of the test water according to this Working Example 80 is shown in Table 4, and the effective value of the dissolved hydrogen concentration DH is shown in FIG. 40.

(Working Example 81)

Using catalyst-free circulating electrolyzed water as test water, which is

30 activated charcoal processed water containing Pd colloid prepared in this manner that has been subjected to electrolysis processing using a continuous flow circulating method (volume of circulatory water: 0.8 liters) for three minutes under the same conditions as those of Working Example 79, 1 mL of one-fortieth strength Pt standard solution that has undergone the nitrogen gas replacement described above is injected to 200 mL of

this test water in a test water holding compartment using a syringe. This is then sufficiently stirred and mixed, and thereafter while visually observing the color change of the test water, a 10 g/L concentration (mole concentration by volume: 26773.8 μM) of methylene blue solution is injected a little bit at a time into the test water using a

5   syringe. The total amount of methylene blue solution injected until reaching the end point was 10.6 mL, and the measured dissolved hydrogen concentration DH found by substituting each value into Equation 7 was 2.84 (mg/L). Each physical property value of the test water according to this Working Example 81 is shown in Table 4, and the effective value of the dissolved hydrogen concentration DH is shown in FIG. 40.

10   (Working Example 82)

Pd colloid-containing activated charcoal processed water, which is obtained by adding the same Pd standard solution as that of Working Example 31 sufficient to give a concentration of 192 μg/L to activated charcoal processed water resulting from processing Fujisawa City tap water through an activated charcoal column, is prepared.

15   Using pre-electrolysis catalyst-added circulating electrolyzed water as test water (AOW), which is Pd colloid-containing activated charcoal processed water prepared in this manner that has been subjected to electrolysis processing using a continuous flow circulating method (volume of circulatory water: 0.8 liters) for three minutes under the same conditions as those of Working Example 80, 1 mL of one-fortieth strength Pt

20   standard solution that has undergone the nitrogen gas replacement described above is injected to 200 mL of this test water in a test water holding compartment using a syringe. This is then sufficiently stirred and mixed, and thereafter while visually observing the color change of the test water, a 10 g/L concentration (mole concentration by volume: 26773.8 μM) of methylene blue solution is injected a little bit at a time into

25   the test water using a syringe. The total amount of methylene blue solution injected until reaching the end point was 12.0 mL, and the measured dissolved hydrogen concentration DH found by substituting each value into Equation 7 was 3.21 (mg/L). Each physical property value of the test water according to this Working Example 82 is shown in Table 4, and the effective value of the dissolved hydrogen concentration DH is

30   shown in FIG. 40.

[Table 4]

★

Examination of influence of antioxidant-functioning water (AOW) upon life span of nematode Caenorhabditis elegans (C. elegans)

Caenorhabditis elegans (hereafter, referred to as 'C. elegans') is a type of Nematoda, and is used worldwide as an aging model for multicellular organisms as well as for fruit-flies, mice, and rats. In addition, the complete genome sequence has been determined in C. elegans, and it has been noticed as a 'living test tube' for examining
5   individual function/ action mechanisms of human hereditary disease-causing genes or oncogenes by combining methods such as gene disruption, expression analysis using GFP fusion genes, and the like.

It is worth noting that the longest life span of wild-type C. elegans is extremely short, approximately 25 days (see 'AGING AT A MOLECULAR LEVEL' written by
10   Naoaki ISHII, Kodansha, 2001, p102-103). Use of C. elegans as an experimental animal allows quick examination of the influence of antioxidant-functioning water upon the life span of animals.

Therefore, the inventors carried out testing in order to examine the influence upon the life span of C. elegans when antioxidant-functioning water (AOW) is used as
15   feeding water for wild C. elegans through the supervision and cooperation of Mr. Naoaki Ishii, assistant professor of molecular life science, School of medicine, Tokai University, who wrote the document 'Aging at a Molecular Level' described above. What is called 'feeding water' here is water that is used during each manipulation of leaving larvae in water for 2 to 3 hours in step (4), and dripping water on the surface of an agar culture
20   when determining either the life or death of the larvae, or when the agar culture becomes dry in step (8) from testing procedures in section (A-2) to be described later, which are carried out for testing groups of purified water and antioxidant-functioning water, respectively.

An outline of this testing protocol is described in the following section (A),
25   working examples and reference examples of this test are described in section (B), and results and examination of the test are described in section (C). It should be noted that this test conforms to 'Section 2, Measurement of life span at various oxygen concentrations' described in P290-292 of 'Aging Model', which is described in P288-292 of 'Active oxygen experimental protocol – measurement method, gene analysis, and
30   pathologic physiological model' edited by Naoyuki Taniguchi: Cell Engineering Separate Volume, Experimental protocol series, issued by Shujunsha, Co., Ltd. (hereafter, this reference is abbreviated as 'reference procedure'.) The contents described in the above-mentioned 'Aging Model' are incorporated herein by reference. It should be noted that a part of the reference procedure is altered in consideration of special

characteristics of this test such as examining the influence upon C. elegans when feeding water is changed. Therefore, in the description of this testing protocol, the altered portions of the reference procedure are mainly described.

(A) Outline of Testing Protocol

5   (A-1) Reagents to be used

Reagents to be used in this test are as follows.

(1) 5-fuluoro-2'-deoxyuridine (FudR) manufactured by Wako Pure Chemical Industries, Ltd.

(2) S buffer

10   Sodium chloride: NaCl (0.1M)

Potassium phosphate (pH6.0)

Since C. elegans are hermaphrodites, a measure is necessary to prevent from getting confused with the next generation. Therefore, reagent FudR is used in conformity with the reference procedure in order to inhibit the next generation of C.

15   elegans from emerging. In addition, the S buffer is used to eliminate influences due to difference in pH.

(A-2) Testing procedures

(1) Wild-type primary larvae that have been subjected to synchronized culture are collected in conformity with the reference procedure. 500 to 1000 larvae are placed in a

20   9 cm schale including the agar culture. The lifetime of larvae upon this manipulation is approximately four days.

(2) A suitable amount of the S buffer that has been collected by a Pasteur's pipette is poured into the above-mentioned schale, and is then suctioned in by the Pasteur's pipette together with the larvae in the schale. The suctioned S buffer and larvae are

25   then transferred to a tube (diameter: 1.5 mm). When the tube is left standing upright, the larvae begin to settle at the bottom of the tube. After the larvae have settled, the S buffer (supernatant) in the tube is gently suctioned and removed using the Pasteur's pipette while being careful not to suction the larvae. As a result, the S buffer in the tube is removed and the larvae are collected in the tube.

30   (3) A suitable amount (approximately 100 to 200) of larvae collected in the tube in step (2) are taken out, and are divided and put into two tubes (diameter: 1.5 mm, capacity: approximately 1 to 2 cc), respectively. A control (purified water of Reference Example 28 to be described later) is then poured into one of the above-mentioned two tubes, and test water (antioxidant-functioning water of Working Example 83 to be described later)

into the other.

(4) Lids are attached to the openings of both tubes, and the tubes are laid down and left to stand for 2 to 3 hours.

(5) Once they have been laid down and left to stand, the larvae are transferred from the respective tubes into two 9 cm schales including the agar culture, and are left to stand overnight at room temperature.

(6) Twenty 3 cm schales including the agar culture with slightly dried surfaces are prepared. One drop of colon bacillus, which is food for the larvae, is applied at nearly the center of each 3-cm schale. Thus, it becomes easy to observe the larvae since they gather to the center to seek for food.

(7) The larvae are transferred from the two respective 9-cm schales that have been left to stand overnight at room temperature in step (5) into ten of the 3-cm schales prepared in step (6) using a platinum wire for isolation to be described later, with ten larvae in each schale. These 100 larvae are used as one test group. When there are two test groups, this manipulation must be carried out for each test group. The reagent FudR is also added when dividing the larvae. Here, the platinum wire for isolation is a tool of the inventors own making where a platinum wire having a length of approximately 3 cm, and diameter of 50 to 100 μm is attached to a narrow-mouthed section of the 3 cm Pasteur's pipette, the tip of the platinum wire is sharpened into an acute angle using a file or the like, and the wire is bent at a right angle approximately 5 mm from the tip. The tip (the portion that touches larvae or agar culture) is sterilized by a burner flame each time it is used. It is used by scooping up a larva from the bottom. When the tip is made to lightly touch the agar after the larva is caught, the larva shifts to the agar culture by itself.

(8) Life and death (lifetime) of the larvae in each group are examined every day (every other day in this test as a rule). In determination of either life or death of the larvae, a larva is determined to be dead when it does not react when stimulated by lightly touching the head of the larva using the platinum wire for isolation or applying a drop of water. When the agar culture becomes dry, several drops of water are applied onto the surface of the agar culture using the Pasteur's pipette.

(B) Disclosure of working examples and reference examples
(Reference Example 28)

Life span data of a nematode C. elegans in conformity with testing procedure described in (A-2) when activated charcoal processed water (purified water) resulting

from processing Fujisawa City tap water through an activated charcoal column is employed as feed water is given as Reference Example 28.

(Working Example 83)

Life span data of a nematode C. elegans obtained in conformity with the testing procedures described in (A-2) when pre-electrolysis catalyst-added circulating electrolyzed water (AOW) is used as feed water, which is obtained by adding an amount of the Pd standard solution described in Working Examples 6 through 8 sufficient to give a Pd colloid concentration of 192 µg/L to 1 liter of the same activated charcoal processed water (purified water) as that of Reference Example 28 that has been subjected to electrolysis processing (corresponding to two-pass electrolysis processing) using a continuous flow circulating method (volume of circulatory water: 0.8 liters) for 1 minute under the conditions of a 1 L/min flow and 5A constant current, is given as Working Example 83.

(C) Test results and examination thereof

FIGS. 41 and 42, which compare Reference Example 28 (a group that uses purified water as feed water) and Working Example 83 (a group that uses antioxidant-functioning water as feed water), show the influence of Pt colloid catalyst-containing electrolyzed water (AOW) upon the life span of C. elegans. In addition, Table 5 shows the results of a significant difference test for differences in average life span between two independent groups according to this test using a student's t-test.

[Table 5]

★

As shown in Table 5, since the result is t = 4.03 > two-sided boundary values for t (3.34) based on the relative risk of 0.1%, the null hypothesis that 'average life spans of a group that uses antioxidant-functioning water as feed water and a group that uses purified water as feed water are equal to each other' is rejected. Accordingly, the average life span (20.05 days) of a group that uses antioxidant-functioning water as feed water (number of samples: 99) is 2.3 days longer than the average life span (17.75 days) of a group that uses purified water as feed water (number of samples: 95). This is a significant difference (t (192) = 4.03, SD = 0.57, and P < 0.001). Here, 't(192) = 4.03' represents the t value, where '192' represents a degree of freedom, SD represents the standard deviation of differences in average life spans of the two groups, and p represents relative risk.

To examine the test results, the life span of the nematode C. elegans has been prolonged since antioxidant-functioning water controls the oxidizing disturbance emanating from active oxygen species. The active oxygen species have toxic effects upon living cells due to oxidizing disturbance to intracellular molecules such as protein,

5 nucleic acid, and the like. In short, it has been clarified through a study using Nematoda and fruit-flies (See references such as 'Life Span Determination Mechanism of Nematoda' Naoaki ISHII, Cell Engineering, vol. 21 No. 7, 2002, Agarwal, S. et al, Proc. Natl. Acad. Sci. U.S.A., 91, 12332-12335, 1994, Larsen, P. L. Proc. Natl. Acad. Sci. U.S.A., 90, 8905-8909, 1993, Sohal, R. S. et al, J. Biol. Chem., 270, 15671-15675, 1995)

10 that the oxidizing disturbance emanating from the active oxygen species is involved in aging. It is certain that in this test as with the contents of these studies, findings suggesting that the oxidizing disturbance emanating from the active oxygen species is involved in aging have been obtained.

Does antioxidant-functioning water (AOW) scavenge the hydroxy radical ($\cdot$OH)?

15 The hydroxy radical ($\cdot$OH) has strong oxidizing power, and is known to extensively damage living organisms by breaking a DNA strand in a living organism, inducing lipoperoxidation, and the like. In other words, how to control the amount of generated hydroxy radical ($\cdot$OH) is important for the living organism.

Regarding the question 'Does antioxidant-functioning water (AOW) scavenge

20 the hydroxy radical ($\cdot$OH)?', scavenging activity of the hydroxy radical ($\cdot$OH) generated by exposing hydrogen peroxide to ultraviolet radiation using a spin trap ESR method is evaluated based on four test sample specimens described later. The spin trap ESR method is a measurement method allowing selective sensitive detection of radical species (active oxygen, transition metal, organic radical, and the like) with an unpaired

25 electron using a system of measurement including a combination of an electron spin resonance (ESR) apparatus and a spin trap reagent. In the following, an outline of the measurement procedure is given.

(A) Outline of Measurement Procedure

(A-2) Specimens

30 Specimens to be provided for this measurement are as follows.

(1) Specimen 1: distilled water that has undergone the nitrogen gas replacement through bubbling

(2) Specimen 2: distilled water that has undergone the nitrogen gas replacement through bubbling

(3) Specimen 3: distilled water that has undergone the nitrogen gas replacement (AOW) through bubbling after inclusion of a Tanaka Kikinzoku-manufactured Pd colloid (particle size distribution is 2 to 4 nm, including polyvinylpyrrolidone (PVP) as a dispersion agent) in approximately 200 μg/L concentration

(4) Specimen 4: distilled water that has undergone the nitrogen gas replacement through bubbling after inclusion of the same Tanaka Kikinzoku-manufactured Pd colloid in approximately 200 μg/L concentration as with specimen 3

(A-2) Reagents used

Reagents used in this measurement are as follows.

(1) 30% hydrogen peroxide solution manufactured by Wako Pure Chemical Industries, Ltd.

(2) 5,5-Dimethyl-1-pyrroline-N-oxide: (DMPO) manufactured by Nacalai Tesque, Inc.

(A-3) Apparatus used

Apparatus used in this measurement are as follows.

(1) ESR spectrometer: ESP350E manufactured by BRUKER

(2) Auxiliary equipment:

i. Microwave Frequency Counter: HP5351 manufactured by Hewlett-Packard Company

ii. Gaussmeter: ER035M manufactured by BRUKER

(A-4) Measurement procedures

(1) Solution preparation:

Preparation of a solution to be mixed with the specimens is carried out completely within a nitrogen gas atmosphere. Pure water described next is used after undergoing nitrogen gas bubbling. Containers used for the specimens and solutions are one-mark pipettes (0.5 mL, 1.0 mL) and volmetric flasks (10 mL, 50 mL).

(2) 1 mM hydrogen peroxide solution:

i. 0.5 mL of 30% hydrogen peroxide solution (9.8M) is collected and diluted with the pure water to make 50 mL, preparing a 100 mM solution.

ii. 0.5 mL of the above-given solution in step i is collected and diluted with the pure water to make 50 mL, preparing a 1 mM solution. Aluminum foil is wrapped around to shield from light when storing.

(3) ESR measurement:

i. 1 mL of the hydrogen peroxide solution (H_2O_2) prepared into 1mM and 15 μL of DMPO is collected and then diluted with a specimen, making 10 ML in total. In this

case, H_2O_2 concentration is 0.1 mM, and DMPO concentration is 13 mM.

ii. The above-given solution of step i is suctioned into an ESR flat cell and measured while irradiating with ultraviolet rays. An ultrahigh pressure mercury lamp (manufactured by Ushio Inc.) is used for ultraviolet ray irradiation through a water

5 filter.

(4) Measurement conditions:

Measurement temperature room temperature

Magnetic field sweep range 3440 to 3450 G

Modulation 100 kHz, 1G

10 Microwave 9.80 GHz, 16 mW

Sweeping period 41.943 s × 1 time

Time constant 81.92 ms

Data point number 1024 points

Cavity TM_{110}, cylindrical

15 (B) Measurement results and examination thereof

·OH radical adduct of DMPO (DMPO-OH) is observed in the four specimens 1 through 4. This is because a hydroxy radical (·OH) deriving from the hydrogen peroxide generated through the ultraviolet ray irradiation of the specimens 1 through 4 is captured by the DMPO, generating DMPO-OH. DMPO-OH relative intensities

20 obtained from an ESR spectrum after 60 seconds has passed since the beginning of ultraviolet ray irradiation onto the specimens 1 through 4 are given in Table 6.

[Table 6]

★

Through comparison of specimen 1 (relative intensity: 1) and specimen 2

25 (relative intensity: 0.29), and specimen 3 (relative intensity: 0.10) and specimen 4 (relative intensity: 0.90), respectively, intense hydroxy radical (·OH) scavenging activity, which may emanate from the dissolved hydrogen within the specimens 2 and 3, is observed. This may result from an electron being extracted from the molecular hydrogen due to the strong oxidation inherent in the hydroxy radical (·OH). In

30 addition, through comparison of specimen 2 (relative intensity: 0.29) and specimen 3 (relative intensity: 0.10), intense hydroxy radical (·OH) scavenging activity regarded to emanate from a combination of the hydrogen within specimen 3 and a palladium colloid is observed. This may result from reducing power, which emanates from the combination of the hydrogen and the palladium colloid, acting on the hydroxy radical

(·OH).

From the above-given measurement results, it has become evident that the antioxidant-functioning water (AOW) scavenges the most oxidative hydroxy radical (·OH) of the active oxygen species.

5 Does antioxidant-functioning water (AOW) control oxidation of reduced vitamin C?

Vitamin C is a water-soluble vitamin, ascorbic acid or reduced vitamin C has strong reducing power, scavenging an active oxygen species such as a superoxide anion radical (·O^{2-}) in the living organism, and further, deoxidizing oxidized vitamin E. However, if the ascorbic acid (reduced vitamin C) is oxidized by coming in contact with

10 oxygen, it changes to monodehydroascorbic acid and then changes to dehydroascorbic acid (oxidized vitamin C). This oxidized vitamin C does not demonstrate reducing power in living organisms. In other words, it is important that vitamin C is kept in a reduced state when ingested by a living organism. Therefore, a test for answering 'Does antioxidant-functioning water (AOW) control oxidation of reduced vitamin C?' is

15 conducted assuming a reduced vitamin C-containing AOW product resulting from inclusion of reduced vitamin C in antioxidant-functioning water (AOW). An outline of a testing protocol is given forthwith.

(A) Outline of Testing Protocol

(A-1) Reagents used

20 Reagents used in this measurement are as follows.

(1) Distilled water manufactured by Wako Pure Chemical Industries, Ltd.

(2) L(+)-ascorbic acid special grade chemical manufactured by Wako Pure Chemical Industries, Ltd.

(3) pH buffer solution: Tris-HCl (7.4), Tris-HCl (9.0), Glycine-HCl (2.2)

25 (A-2) Analyzers used

Apparatus used in this measurement are as follows.

(1) UV/Visible Spectrophotometer: Ultrospec 3300 *pro* manufactured by Amersham Pharmacia Biotech Inc.

(2) Auxiliary equipment: thermo-cell holder manufactured by Amersham Pharmacia

30 Biotech Inc.

(A-3) Solution preparation

(1) Add 50 mg ascorbic acid (AsA) to 100 mL nitrogen gas-replaced distilled water to make an aqueous ascorbic acid solution. However, since the ascorbic acid aqueous solution gradually oxides when left in an atmospheric corrosive environment, aqueous

ascorbic acid solution is prepared and used for each test.

(2) pH buffer solution; distilled water containing 100 mM of Tris-HCl (7.4), distilled water containing 100 mM of Tris-HCl (9.0), and distilled water containing 100 mM of Glycine-HCl (2.2) are respectively prepared as distilled water with respective pHs,

5 and are called distilled water (7.4), distilled water (9.0), and distilled water (2.2), respectively. Meanwhile, the respective distilled waters prepared to the respective pHs having undergone hydrogen gas replacement are made into hydrogen water with the respective pHs and are called hydrogen water (7.4), hydrogen water (9.0), and hydrogen water (2.2), respectively.

10 (3) To prepare antioxidant-functioning water by including a precious metal colloid catalyst in the hydrogen waters prepared with the respective pHs of the above step (2), a precious metal colloid catalyst (including any one of four types: Pt, Pd, Pt/Au alloy, or Pd/Au alloy, which are all manufactured by Tanaka Kikinzoku, have a particle size distribution of 2 to 4 nm, and include polyvinylpyrrolidone (PVP) as a dispersion agent)

15 is added, and hydrogen gas replacement is then carried out.

(A-4) Testing procedures

(1) An ALDRICH-manufactured rubber stopper is inserted in each quartz cell of a spectrophotometer (optical length: 1 cm, capacity: 3cc), and the air in the cell is replaced with hydrogen gas.

20 (2) 2 mL of the antioxidant-functioning water prepared in (A-3) - (3) above is collected in a plastic syringe and then injected in a quartz cell.

(3) 100 μL of the ascorbic acid aqueous solution prepared in (A-3) - (1) described above is collected in a plastic syringe and then injected in another quartz cell. At this time, the ascorbic acid concentration within the quartz cell is 135 μm.

25 (4) The quartz cell is quickly set in the spectrophotometer, and changes in absorption wavelength 250 nm peculiar to reduced vitamin C over time during 30 minutes, namely, changes in absorbency (A250) during the period from 0 to 1800 minutes, and absorption spectrum every thirty minutes are measured and recorded. It should be noted that temperature inside all quartz cells is 37°C due to the thermo-cell holder.

30 (5) During measurement, oxygen (air) is gradually mixed into the quartz cell through the ALDRICH-manufactured rubber stopper. This allows oxidation of the reduced vitamin C to progress.

(6) Test results are represented by residual ratios (%) of the reduced vitamin C at every 30 minutes since the beginning of the test. It should be noted that as the

residual ratio (%) of the reduced vitamin C is found, correction of subtracting the amount of absorbency emanating from oxidized vitamin C from the measured absorbency is carried out.

How to find the residual ratio (%) of reduced vitamin C and the basis for absorbency correction are given forthwith.

First, to describe how to find the residual ratio (%) of reduced vitamin C, the maximum absorption wavelength in the ultraviolet range for reduced vitamin C (AsA) shifts depending on the liquidity of the solution. More specifically, it shifts to the vicinity of 240 nm in an acidic range, and to the vicinity of 270 nm from neutral to basic ranges. With this test, 250 nm, which allows characteristic detection in all liquidity ranges, is selected as the reduced vitamin C absorption wavelength.

The reduced vitamin C residual ratio (%) at the wavelength 250 nm selected as described above is found through a calculation expression $[A250(T) - A250(min.)]/[A250(0)- A250(min.)]$, where $T= 0, 30, 60, 90, 120, 150, 180$, and $A250(min.) = 0.311$. It should be noted that when absorption of the reduced vitamin C (AsA) has stopped, there is still oxidized vitamin C on the whole to be absorbed, which is represented as $A250(min.) = 0.311$. Incidentally, $A250(min.)$ also includes the absorption spectrum of distilled water.

Next, to describe the basis for this absorbency correction, the specific absorbance spectrum in the ultraviolet range of reduced vitamin C (AsA) emanates from the enediol group included in the reduced vitamin C (AsA). The enediol group forms a reduced vitamin C (AsA) conjugate structure, which is an essential element for generating the specific absorbance spectrum in the ultraviolet range. The enediol group has two hydrogen atoms, which develop the reducing power of the reduced vitamin C.

Meanwhile, vitamin C having lost both hydrogen atoms from the enediol group changes to oxidized vitamin C (DHA), and then further changes to an oxidized decomposition product. Since the enediol group is lost at this time, the specific absorbance in the ultraviolet range is gone as a result of losing the conjugated structure.

This means that the specific absorbance spectrum of the reduced vitamin C (AsA) is gone, and not that the entire vitamin C structure is completely gone. In other words, there is oxidized vitamin C left, and this does not mean that the vitamin C is completely decomposed into water and carbon dioxide and then disappears without a

trace. The absorption spectrum of oxidized ascorbic acid (DHA) may be thought as resulting from subtraction of the enediol group's contribution from the absorption spectrum of the reduced vitamin C (AsA).

When reduced vitamin C (AsA) and oxidized vitamin C (DHA) coexist, the molar absorptivity □of the reduced vitamin C (AsA) is greater than that of the oxidized vitamin C (DHA), and thus the absorption spectrum of the reduced vitamin C (AsA) does not hide behind the absorption spectrum of the oxidized vitamin C (DHA). Therefore, even if the amount of the absorption spectrum of the oxidized vitamin C (DHA) is subtracted from the absorption spectrum of the reduced vitamin C (AsA), since the peculiar absorption spectrum of reduced vitamin C still remains, there is no adverse affect on accuracy of the reduced vitamin C residual ratio (%) even if absorbency correction is carried out as with this test.

(B) Disclosure of working examples and reference examples

(Reference Example 29)

Reduced vitamin C residual ratio measurement data obtained in conformity with the testing procedures described in (A-4) when distilled water (7.4) with the pH prepared in the above-given (A-3) - (2) is employed as test water is given as Reference Example 29.

(Reference Example 30)

Reduced vitamin C residual ratio measurement data obtained in conformity with the testing procedures described in (A-4) when distilled water (9.0) with the pH prepared in the above-given (A-3) - (2) is employed as test water is given as Reference Example 30.

(Reference Example 31)

Reduced vitamin C residual ratio measurement data obtained in conformity with the testing procedures described in (A-4) when distilled water (2.2) with the pH prepared in the above-given (A-3) - (2) is employed as test water is given as Reference Example 31.

(Reference Example 32)

Reduced vitamin C residual ratio measurement data obtained in conformity with the testing procedures described in (A-4) when distilled water (7.4) with the pH prepared in the above-given (A-3) - (2) is employed as test water is given as Reference Example 32.

(Reference Example 33)

Reduced vitamin C residual ratio measurement data obtained in conformity with the testing procedures described in (A-4) when distilled water (9.0) with the pH prepared in the above-given (A-3) - (2) is employed as test water is given as Reference Example 33.

5   (Reference Example 34)

Reduced vitamin C residual ratio measurement data obtained in conformity with the testing procedures described in (A-4) when distilled water (2.2) with the pH prepared in the above-given (A-3) - (2) is employed as test water is given as Reference Example 34.

10   (Working Example 84)

Reduced vitamin C residual ratio measurement data obtained in conformity with the testing procedures described in (A-4) when antioxidant-functioning water is used as test water, which is obtained by adding to hydrogen water (7.4) an amount of the same Pt standard solution described in Working Examples 3 through 5 sufficient to

15   give a colloid concentration of 200 µg/L, is given as Working Example 84.

.(Working Example 85)

Reduced vitamin C residual ratio measurement data obtained in conformity with the testing procedures described in (A-4) when antioxidant-functioning water is used as test water, which is obtained by adding to hydrogen water (9.0) an amount of

20   the same Pt standard solution as in Working Example 84 sufficient to give a colloid concentration of 200 µg/L, is given as Working Example 85.

(Working Example 86)

Reduced vitamin C residual ratio measurement data obtained in conformity with the testing procedures described in (A-4) when antioxidant-functioning water is

25   used as test water, which is obtained by adding to hydrogen water (2.2) an amount of the same Pt standard solution as in Working Example 84 sufficient to give a colloid concentration of 200 µg/L, is given as Working Example 86.

(Working Example 87)

Reduced vitamin C residual ratio measurement data obtained in conformity

30   with the testing procedures described in (A-4) when antioxidant-functioning water is used as test water, which is obtained by adding to hydrogen water (7.4) an amount of the Pd standard solution described in Working Examples 6 through 8 sufficient to give a colloid concentration of 200 µg/L, is given as Working Example 87.

(Working Example 88)

Reduced vitamin C residual ratio measurement data obtained in conformity with the testing procedures described in (A-4) when antioxidant-functioning water is used as test water, which is obtained by adding to hydrogen water (9.0) an amount of the same Pd standard solution as in Working Example 87 sufficient to give a colloid

5   concentration of 200 μg/L, is given as Working Example 88.

(Working Example 89)

Reduced vitamin C residual ratio measurement data obtained in conformity with the testing procedures described in (A-4) when antioxidant-functioning water is used as test water, which is obtained by adding to hydrogen water (2.2) an amount of

10   the same Pd standard solution as in Working Example 87 sufficient to give a colloid concentration of 200 μg/L, is given as Working Example 89.

(Working Example 90)

Reduced vitamin C residual ratio measurement data obtained in conformity with the testing procedures described in (A-4) when antioxidant-functioning water is

15   used as test water, which is obtained by adding to hydrogen water (7.4) an amount of a Tanaka Kikinzoku-manufactured Pt/Au alloy colloid (it is an alloy colloid having a structure with a Pt core and an Au shell, where the Au shell completely covers the Pt core; the Pt/Au metal mole ratio is 3.71/6.29, and the Pt/Au atomicity ratio for a single Pt/Au alloy cluster is 55/92; in other words, the Pt/Au alloy cluster is a positive 20-sided

20   type alloy cluster with a magic number: 147, where the Au shell (92 atoms) completely covers the Pt core (55 atoms)) -containing solution sufficient to give a colloid concentration of 200 μg/L, is given as Working Example 90.

(Working Example 91)

Reduced vitamin C residual ratio measurement data obtained in conformity

25   with the testing procedures described in (A-4) when antioxidant-functioning water is used as test water, which is obtained by adding to hydrogen water (9.0) an amount of the same Pt/Au alloy colloid-containing solution as in Working Example 90 sufficient to give a colloid concentration of 200 μg/L, is given as Working Example 91.

(Working Example 92)

30   Reduced vitamin C residual ratio measurement data obtained in conformity with the testing procedures described in (A-4) when antioxidant-functioning water is used as test water, which is obtained by adding to hydrogen water (2.2) an amount of the same Pt/Au alloy colloid-containing solution as in Working Example 90 sufficient to give a colloid concentration of 200 μg/L, is given as Working Example 92.

(Working Example 93)

Reduced vitamin C residual ratio measurement data obtained in conformity with the testing procedures described in (A-4) when antioxidant-functioning water is used as test water, which is obtained by adding to hydrogen water (7.4) an amount of a

5 Tanaka Kikinzoku-manufactured Pd/Au alloy colloid- (it is an alloy colloid having a structure with a Pd core and an Au shell, where the Au shell completely covers the Pd core; the Pd/Au metal mole ratio is 3.72/6.28, and the Pd/Au atomicity ratio for a single Pd/Au alloy cluster is 55/92; in other words, the Pd/Au alloy cluster is a positive 20-sided type alloy cluster with a magic number: 147, where the Au shell (92 atoms) completely

10 covers the Pd core (55 atoms)) containing solution sufficient to give a colloid concentration of 200 μg/L, is given as Working Example 93.

(Working Example 94)

Reduced vitamin C residual ratio measurement data obtained in conformity with the testing procedures described in (A-4) when antioxidant-functioning water is

15 used as test water, which is obtained by adding to hydrogen water (9.0) an amount of the same Pd/Au alloy colloid-containing solution as in Working Example 93 sufficient to give a colloid concentration of 200 μg/L, is given as Working Example 94.

(Working Example 95)

Reduced vitamin C residual ratio measurement data obtained in conformity

20 with the testing procedures described in (A-4) when antioxidant-functioning water is used as test water, which is obtained by adding to hydrogen water (2.2) an amount of the same Pd/Au alloy colloid-containing solution as in Working Example 93 sufficient to give a colloid concentration of 200 μg/L, is given as Working Example 95.

(C) Test Results

25 FIG. 43, which compares Reference Examples 29, 32, and Working Examples 84, 87, 90 and 93, shows characteristics of the reduced vitamin C residual ratio (%) changing over time when reduced vitamin C is included in various neutral test waters using a buffer solution (pH 7.4). Based on the same diagram, comparing to Reference Example 29 (distilled water) preservation qualities of the reduced vitamin C when the

30 solution is neutral, Working Example 84 (Pt colloid-containing hydrogen water) is particularly superior, and Working Example 90 (Pt/Au alloy colloid-containing hydrogen water), Working Example 93 (Pd/Au alloy colloid-containing hydrogen water), Working Example 87 (Pd colloid-containing hydrogen water), and Reference Example 32 (hydrogen water) show subsequently favorable preservation quality in this order.

FIG. 44, which compares Reference Examples 30, 33, and Working Examples 85, 88, 91 and 94, shows characteristics of the reduced vitamin C residual ratio (%) changing over time when reduced vitamin C is included in various basic test waters using a buffer solution (pH 9.0). Based on the same diagram, comparing to Reference

5 Example 30 (distilled water) preservation qualities of the reduced vitamin C when the solution is basic, Working Example 85 (Pt colloid-containing hydrogen water) and Working Example 88 (Pd colloid-containing hydrogen water) are particularly superior, and Reference Example 33 (hydrogen water), Working Example 94 (Pd/Au alloy colloid-containing hydrogen water), and Working Example 91 (Pt/Au alloy

10 colloid-containing hydrogen water) show subsequently favorable preservation quality in this order.

FIG. 45, which compares Reference Examples 31, 34, and Working Examples 86, 89, 92 and 95, shows characteristics of the reduced vitamin C residual ratio (%) changing over time when the reduced vitamin C is included in various acidic test waters

15 using a buffer solution (pH 2.2). Based on the same diagram, comparing to Reference Example 31 (distilled water) preservation qualities of the reduced vitamin C when the solution is acidic, Working Example 86 (Pt colloid-containing hydrogen water), Working Example 89 (Pd colloid-containing hydrogen water), and Working Example 95 (Pd/Au alloy colloid-containing hydrogen water) are particularly superior, and Reference

20 Example 34 (hydrogen water) and Working Example 92 (Pt/Au alloy colloid-containing hydrogen water) show subsequently favorable preservation quality in this order.

(D) Examination of results

To begin with, the obtained results are examined in general. Compared to Reference Examples 19 through 31 (distilled water) in all of the liquidity ranges:

25 neutral, acidic, and basic, the other examples showed favorable preservation qualities. One of the reasons may be that the concentration of dissolved oxygen with the other examples being less than those of the Reference Examples 19 through 31 (distilled water) has worked advantageously in terms of controlling oxidation of reduced vitamin C. Furthermore, an acidic liquid can be seen as advantageous for enhancing

30 preservation quality of reduced vitamin C. This will emanate from the fact that vitamin C is an acid that provides low proton disassociation, thereby preventing electrons from being released. On the contrary, a basic liquid provides high proton disassociation, thereby making it easier to release electrons. Therefore, it is understood that the liquidity of the solution preferably leans toward acidity to increase

preservation quality of the reduced vitamin C contained in the aqueous solution.

Next, the obtained results are each critically examined. First, preservation quality of reduced vitamin C in the neutral range in Working Example 84 (Pt colloid-containing hydrogen water) is overwhelmingly superior to that in Reference

5 Example 32 (hydrogen water). Why? What is similar between Working Example 84 (Pt colloid-containing hydrogen water) and Reference Example 32 (hydrogen water) is that the concentration of dissolved oxygen is low at least right after electrolysis. In other words, in both examples, oxidation of the reduced vitamin C progressing over time results from the action of the oxygen being mixed into the quartz cell over time, and this

10 degree of mixing in oxygen over time shows hardly any difference therebetween. Should this be the case, the similarity between these two examples is inferable that whether or not a precious metal catalyst is contained contributes to the preservation quality of the reduced vitamin C. Therefore, the presumed action mechanism thereof is as follows.

15 When two molecules of reduced vitamin C (AsA) undergo single electron oxidation with one molecule of oxygen, two radical molecules of monodehydroascorbic acid (MDA·) and one molecule of hydrogen peroxide are generated. It should be noted that when one molecule of reduced vitamin C (AsA) undergoes single electron oxidation with one molecule of oxygen, one molecule of monodehydroascorbic acid (MDA·) and one

20 molecule of superoxide anion radical ($\cdot O_2^-$) may be generated.

$2AsA + O_2 \square 2MDA\cdot + H_2O_2$

$(AsA + O_2 \square MDA\cdot + \cdot O_2^-)$

Furthermore, two molecules of monodehydroascorbic acid (MDA·) generates reduced vitamin C (AsA) and dehydroascorbic acid (DHA) through a disproportionation

25 reaction. Dehydroascorbic acid (DHA) is a 2-electron oxide of reduced vitamin (AsA).

$MDA\cdot + MDA\cdot \square AsA + DHA$

Through such process, the reduced vitamin C (AsA) is gradually oxidized, and all of the reduced vitamin C (AsA) is ultimately changed into dehydroascorbic acid (DHA). Such process is as described earlier, where liquidity is controlled the more it

30 leans to the acidic side.

Furthermore, examination of reactivity between dehydroascorbic acid (DHA) and Working Example 84 (Pt colloid-containing hydrogen water), and reactivity between dehydroascorbic acid (DHA) and Reference Example 32 (hydrogen water) as a pilot study for this test has revealed that there was no reaction at all therebetween.

This means that once oxidized to dehydroascorbic acid (DHA), it can no longer return to being reduced vitamin C (AsA) using hydrogen molecules regardless of whether or not a precious metal catalyst is contained therein.

As shown in FIG. 43, reactivity between the reduced vitamin C (AsA) and an oxygen molecule is quite high. Furthermore, reactivity between the reduced vitamin C (AsA) and the superoxide anion radical ($\cdot O_2^-$) is well known to also be quite high. In this case, the superoxide anion radical ($\cdot O_2^-$) undergoes single electron oxidation, into hydrogen peroxide.

Therefore, a reaction generating the above-given hydrogen peroxide may be employed as a representative reaction between the reduced vitamin C (AsA) and oxygen molecule.

Here, a case where an oxygen molecule is being mixed into the water of Working Example 84 (Pt colloid-containing hydrogen water) over time is assumed. In this case, two-molecules of reduced vitamin C (AsA) undergo single electron oxidation with one molecule of oxygen, generating two radical molecules of monodehydroascorbic acid (MDA$\cdot$) and one molecule of hydrogen peroxide.

At this time, Working Example 84 (Pt colloid-containing hydrogen water) may subject the monodehydroascorbic acid (MDA$\cdot$) to single electron reduction, returning to the reduced vitamin C (AsA) before two molecules of monodehydroascorbic acid (MDA$\cdot$) initiate a disproportionation reaction. As a result, generation of the dehydroascorbic acid (DHA) may be delayed.

$$MDA\cdot + (H_2 + Pt) \ \square \ AsA + (H\cdot + Pt)$$
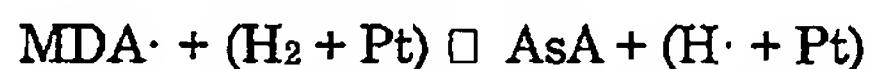
In proportion to the delayed period of dehydroascorbic acid (DHA) generation, the period of maintaining the state as reduced vitamin C (AsA) increases. This can be the reason for the preservation quality of reduced vitamin C in Working Example 84 (Pt colloid-containing hydrogen water) being superior to that in the Reference Example 32 (hydrogen water).

It should be noted that with Reference Example 32 (hydrogen water), since single electron reduction of monodehydroascorbic acid (MDA$\cdot$) cannot occur at all or can occur very little, delay in dehydroascorbic acid (DHA) generation may be impossible.

Meanwhile, the reason why platinum (Pt) is especially superior as a precious metal catalyst for enhancing the preservation quality of the reduced vitamin C (AsA) in the neutral range may be as follows.

First, as mentioned before, when the reduced vitamin C (AsA) undergoes single

electron oxidation with a molecule of oxygen, monodehydroascorbic acid (MDA·) radicals and hydrogen peroxide are generated.

$$2AsA + O_2 \ \Box \ 2MDA· + H_2O_2$$

The Working Example 84 (Pt colloid-containing hydrogen water) has extremely

5 high reactivity with the hydrogen peroxide generated at this time, and can quickly reduce to water. Furthermore, the Working Example 84 (Pt colloid-containing hydrogen water) has extremely high reactivity with the oxygen molecule mixed therein, and can quickly reduce to hydrogen peroxide or water.

$$O_2 + (H_2 + Pt) \ \Box \ H_2O_2 + Pt$$

10 $$H_2O_2 + (H_2 + Pt) \ \Box \ 2(H_2O) + Pt$$
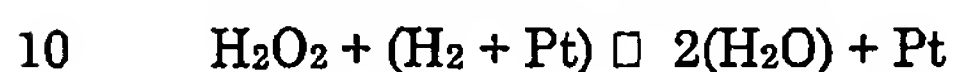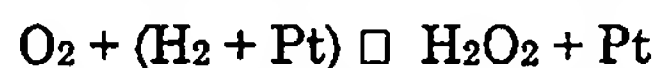
Meanwhile, the precious metal catalysts contained in Working Example 90 (Pt/Au alloy colloid-containing hydrogen water), Working Example 93 (Pd/Au alloy colloid-containing hydrogen water), and Working Example 87 (Pd colloid-containing hydrogen water) may have lower reactivity with oxygen and with hydrogen peroxide

15 than platinum (Pt).

However, the reduced vitamin C (AsA) can be maintained for a longer duration with Working Example 90 (Pt/Au alloy colloid-containing hydrogen water), Working Example 93 (Pd/Au alloy colloid-containing hydrogen water), and Working Example 87 (Pd colloid-containing hydrogen water) than with Reference Example 32 (hydrogen

20 water). This is because there is hardly any promise of reactivity with oxygen molecules and hydrogen peroxide with the Working Examples 90, 93, and 87, while they have nearly the same action with monodehydroascorbic acid (MDA·) as does Working Example 84 (Pt colloid-containing hydrogen water).

In other words, this means that in general, hydrogen water containing a

25 precious metal colloid (antioxidant-functioning water) can quickly reduce oxygen or hydrogen peroxide to water before reduced vitamin C (AsA) is oxidized to oxygen or hydrogen peroxide, and can control generation of dehydroascorbic acid (DHA) and monodehydroascorbic acid (MDA·).

Accordingly, the hydrogen water containing a precious metal colloid

30 (antioxidant-functioning water) is used for improving the preservation quality of the reduced vitamin C (AsA), which is preferred.

Next, Working Example 94 (Pd/Au alloy colloid-containing hydrogen water) and Working Example 91 (Pt/Au alloy colloid-containing hydrogen water) are weaker in preservation quality of reduced vitamin C in the basic range than Reference Example

33 (hydrogen water). Why? This is because the Pd/Au alloy colloid or Pt/Au alloy colloid contained in Working Example 94 or Working Example 91 is regarded as enhancing reactivity between the reduced vitamin C (AsA) and oxygen molecules irrelevant to the oxygen, or hydrolysis of the reduced vitamin C (AsA) acts as a catalyst instead of oxidative decomposition.

In addition, Working Example 92 (Pt/Au alloy colloid-containing hydrogen water) is weaker in preservation quality of reduced vitamin C in the acidic range than Reference Example 34 (hydrogen water). Why? This is because the Pt/Au alloy colloid contained in Working Example 92 is regarded as enhancing reactivity between the reduced vitamin C (AsA) and oxygen molecules irrelevant to the oxygen, or hydrolysis of the reduced vitamin C (AsA) acts as a catalyst instead of oxidative decomposition.

Does antioxidant-functioning water (AOW) control lipoperoxidation?

Lipid peroxide denotes a toxic substance generated when a free radical included in an active oxygen species joins to an unsaturated fatty acid (mostly contained in vegetable oil and fish fat) within a living organism. It has been found that this lipid peroxide is prominently responsible for hepatic/renal damage, ischemia/reperfusion injury, circulatory system diseases such as arteriosclerosis, gastric ulcers, digestive system diseases such as gastric mucosa injury, respiratory diseases, diabetes complications (e.g., high blood pressure, cerebral infarction, heart attack, and the like), cataract, cutaneous diseases, various inflammatory diseases, neurological disorders, cancer, aging, and the like. How to control lipoperoxidation is extremely important for living organisms.

Accordingly, a pharmacological test is conducted using seven groups of 10 rats/group regarding the question 'Does antioxidant-functioning water (AOW) demonstrate pharmacologic function such as control generation of lipid peroxide in rats?' when consumption of antioxidant-functioning water (AOW) is assumed. An outline of a testing protocol is given forthwith.

(A) Outline of Testing Protocol

(A-1) Test animals and breeding environment

Six week-old male specific pathogen free (SPF) rats purchased from CLEA Japan, Inc. were bred in preparation for seven days and then provided for testing. The rats were bred in plastic cages installed in a breeding room (lighting hours: 8 AM to 8 PM, air conditioning: all fresh system) at a room temperature of 24 ± 1°C and humidity

of 55 ± 5% throughout the preparatory breeding period and the testing period.

Regarding feed, all groups were freely given solid feed (CE-2 manufactured by CLEA Japan, Inc.). Regarding water supply, distilled water was filled into feedwater bottles and freely taken in during the preparatory breeding period. Once testing began,

5 test articles were filled into respective feedwater bottles and freely taken in.

(A-2) Main instruments, equipment and materials, and reagents used in test

(1) Refrigerated Centrifuge: type 5930 manufactured by KUBOTA Corporation

(2) Homogenizer: HG-92G manufactured by TAITEC Corporation

(3) UV/Visible Spectrophotometer: Ultrospec 3100 *pro* manufactured by Amersham

10 Pharmacia Biotech Inc.

(4) Radical initiator (AAPH: 2,2-Azobis-amidinopropane dihydrochloride)

(5) 8-OHdG measuring kit: Japan Institute for The Control of Aging

(A-3) Outline of test articles

(1) Purified water (used for Control Groups 1-0, 1-1)

15 Pt colloid catalyst (approximately 200 μg/L concentration) -containing purified water (used for Control Group 1-2)

(3) Pd colloid catalyst (approximately 200 μg/L concentration) -containing purified water (used for Control Group 1-3)

(4) Catalyst-free circulating electrolyzed water (electrolyzed hydrogen water, used for

20 Test Group 1-1)

(5) Pre-electrolysis catalyst-added circulating electrolyzed water (concentration of the Pt colloid catalyst is approximately 200 μg/L) -containing electrolyzed hydrogen water, used for Test Group 1-2)

(6) Pre-electrolysis catalyst-added circulating electrolyzed water (concentration of the

25 Pd colloid catalyst is approximately 200 μg/L) -containing electrolyzed hydrogen water, used for Test Group 1-3)

(A-4) Testing method and testing items

(1) Measurement of weight, feed intake, and water intake

Weight, feed intake, and water intake were measured between 10 and 11 AM

30 daily.

(2) Test article administration method

Immediately after opening a 200 mL capacity light-resistant glass bottle, 2 mL of each test article (200 mL included in that bottle) assigned to the rats in each group was forcedly administered orally to each rat using a disposable gastric sonde for seven

days from the first day of testing to the seventh day. During that period, the water in the feedwater bottles was exchanged with fresh water. In other words, once old water left in the feedwater bottles was discarded, fresh remaining water after being used for forced oral administration was gently filled up to the maximum amount of the feedwater bottles so as to remove as much of the air layer therein as possible, and water supply taps were then set so as to freely provide water. It should be noted that forced oral administration was conducted twice a day between 10 to 11 AM and 5 to 6 PM. In addition, forced oral administration of the test articles was conducted as described above immediately before administration of the radical initiator AAPH (may be referred to as AAPH hereafter.) Water in the feedwater bottles was replaced with fresh water as described above and freely provided until time to dissect.

(3) Preparation of radical initiator AAPH and inducement of lipoperoxidation

Since the AAPH is water soluble, 50 mg/kg B.W. was prepared using physiological saline. Preparation was carried out on the day of AAPH administration. AAPH prepared as such was administered on the seventh day after testing had begun to the abdominal cavities of rats in six groups except for Control Group 1-0 described later, thereby inducing lipoperoxidation. It should be noted that physiological saline was administered to the abdominal cavities for Control Group 1-0.

(4) Urine sample extraction

Urine was extracted using a metabolic cage during a period of twelve hours after AAPH administration until dissection (nothing by mouth during this period). The extracted urine was filtered for impurities and then kept frozen under -80°C until provided for analysis.

(5) Dissection

Once twelve hours had passed (nothing by mouth during this period) after AAPH administration completion, they were dissected under ether anesthesia. After gross examination of the livers, the livers were extracted and kept frozen under -80°C until provided for analysis.

(6) Measurement of urinary 8-OHdG (8-hydroxy-2'-deoxyguanosine) levels

The urine extracted in the above-given (A-4) - (4) was measured for 8-OHdG levels. It should be noted that 8-OHdG is widely used as a marker for oxidative stress.

(7) Measurement of amount of lipid peroxide (TBARS: thiobarbituric acid reactive substances) in liver

After the livers removed in the above-given (A-4) - (5) were thawed, they were

homogenized in ice, and the amount of lipid peroxide (TBARS: thiobarbituric acid reactive substances) in the livers was measured through a thiobarbituric acid (TBA) method. It should be noted that TBARS is widely used as a marker for lipoperoxidation.

5 (8) Statistical processing

With obtained measured data, the mean value ± a standard error was calculated for every group. Student's t-test was conducted to verify statistic significances among the respective groups, where p-value < 0.05 was found to be statistically significant.

10 (B) Disclosure of test groups and control groups

(Control Group 1-0)

A group tested for ten rats in conformity with the testing procedure described in (A-2) when activated charcoal processed water (purified water) resulting from processing Fujisawa City tap water through an activated charcoal column is employed

15 as feed water is given as Control Group 1-0. It should be noted that Control Group 1-0 is a group bred in a normal environment, and physiological saline is administered instead of AAPH to the abdominal cavity. In other words, there is no generation of lipid peroxide with Control Group 1-0.

(Control Group 1-1)

20 A group tested for ten rats in conformity with the testing procedure described in (A-2) when the same purified water as with Control Group 1-0 is used for feeding water is given as Control Group 1-1. It should be noted that the difference between Control Group 1-0 and Control Group 1-1 is whether or not AAPH has been administered.

25 (Control Group 1-2)

A group tested for ten rats in conformity with the testing procedure described in (A-2) when the same purified water as with Control Group 1-0 added an amount of the Pt standard solution described with Working Examples 3 through 5 sufficient to give a Pt colloid concentration of 192 µg/L is used for feeding water is given as Control

30 Group 1-2.

(Control Group 1-3)

A group tested for ten rats in conformity with the testing procedure described in (A-2) when the same purified water as with Control Group 1-0 added an amount of the Pd standard solution described with Working Examples 6 through 8 sufficient to

give a Pd colloid concentration of 192 µg/L is used for feeding water is given as Control Group 1-2. It should be noted that the difference between Control Group 1-2 and Control Group 1-3 is type of precious metal colloid contained therein.

(Test Group 1-1)

5   A group tested for ten rats in conformity with the testing procedures described in (A-4) when catalyst-free circulating electrolyzed water is used as feeding water, which results from subjecting 1 liter of the same purified water as with Control Group 1-0 to electrolysis processing (equivalent to 2-pass electrolysis processing) for one minute using a continuous flow circulating method (volume of circulatory water: 0.8

10   liters) under conditions of a 1.5 L/min flow and 5A constant current, is given as Test Group 1-1.

(Test Group 1-2)

A group tested for ten rats in conformity with the testing procedures described in (A-4) when pre-electrolysis catalyst-added circulating electrolyzed water added an

15   amount of the Pt standard solution described with Working Examples 3 through 5 sufficient to give a Pt colloid concentration of 192 µg/L is used as feeding water, which results from subjecting 1 liter of the same purified water as with Control Group 1-0 to electrolysis processing (equivalent to 2-pass electrolysis processing) for one minute using a continuous flow circulating method (volume of circulatory water: 0.8 liters)

20   under the same conditions of Test Group 1-1, is given as Test Group 1-2.

(Test Group 1-3)

A group tested for ten rats in conformity with the testing procedures described in (A-4) when pre-electrolysis catalyst-added circulating electrolyzed water added an amount of the Pd standard solution described with Working Examples 6 through 8

25   sufficient to give a Pd colloid concentration of 192 µg/L is used as feeding water, which results from subjecting 1 liter of the same purified water as with Control Group 1-0 to electrolysis processing (equivalent to 2-pass electrolysis processing) for one minute using a continuous flow circulating method (volume of circulatory water: 0.8 liters) under the same conditions of Test Group 1-1, is given as Test Group 1-3. It should be

30   noted that the difference between Test Group 1-2 and Test Group 1-3 is the type of precious metal colloid contained therein.

(C) Test Results

Weight, feed intake, and water intake

No differences in weight shift among the seven groups: Control Groups 1-0

through 1-3 and Test Groups 1-1 through 1-3 were recognized in the seven days before AAPH administration. Neither were any differences in feed intake or water intake among the same seven groups recognized in said period.

State observation

5   Regarding state observation seven days before AAPH administration and after AAPH administration, no abnormality was recognized in external appearance or behavior before AAPH administration. On the other hand, after AAPH administration, there were no abnormalities in behavior observed in comparison to before AAPH administration except for fur a little raised in each group.

10   Urinary 8-OHdG level

        FIG. 46 and Table 7, which compare seven groups: Control Groups 1-0 through 1-3 and Test Groups 1-1 through 1-3 for urinary 8-OHdG levels, show the influence of consumption of precious metal colloid (Pt or Pd) catalyst-containing electrolyzed hydrogen water (AOW) upon control of rat DNA oxidation damage.

15   [Table 7]

    ★

        The urinary 8-OHdG levels given in FIG. 46 and Table 7 show significantly low values for the three groups: Test Group 1-1 (electrolyzed hydrogen water consuming group), Test Group 1-2 (Pt colloid catalyst-containing electrolyzed hydrogen water

20   consuming group), and Test Group 1-3 (Pd colloid catalyst-containing electrolyzed hydrogen water consuming group) in comparison to Control Group 1-1 (purified water consuming group). In addition, significantly low values were shown for the two groups: Test Groups 1-2 and 1-3 in comparison to the two groups: Control Group 1-2 (Pt colloid catalyst-containing purified water consuming group) and Control Group 1-3 (Pd colloid

25   catalyst-containing purified water consuming group). Furthermore, the two groups: Test Groups 1-2 and 1-3 showed lower values than Test Group 1-1, but not significant differences. Comparing the two groups: Test Groups 1-2 and 1-3 both showed nearly the same value.

Gross observation of liver

30   Gross observation of the livers removed at the time of dissection recognized resilience in the three groups: Test Groups 1-1 through 1-3 in comparison to the three groups: Control Groups 1-1 through 1-3, and were in nearly normal liver conditions.

Amount of lipid peroxide (TBARS: thiobarbituric acid reactive substances) in liver

        FIG. 47 and Table 8, which compare seven groups: Control Groups 1-0 through

1-3 and Test Groups 1-1 through 1-3 for amount of lipid peroxide in the liver, show the influence of consumption of precious metal colloid (Pt or Pd) catalyst-containing electrolyzed hydrogen water (AOW) upon control of lipoperoxidation in rats.

[Table 8]

5 ★

The amounts of lipid peroxide in the livers given in FIG. 47 and Table 8 show significantly low values for the three groups: Test Group 1-1 (electrolyzed hydrogen water consuming group), Test Group 1-2 (Pt colloid catalyst-containing electrolyzed hydrogen water consuming group), and Test Group 1-3 (Pd colloid catalyst-containing 10 electrolyzed hydrogen water consuming group) in comparison to Control Group 1-1 (purified water consuming group) and Control Groups 102 (Pt colloid catalyst-containing purified water consuming group). In addition, Test Groups 1-2 and 1-3 showed lower values than Test Group 1-1, but not significant differences. Furthermore, Test Group 1-3 showed a lower value than Test Groups 1-2 and 1-3, but 15 not a significant difference.

(D) Examination of results

Forced oral administration of test articles and free intake antioxidant actions were compared and studied using a model animal in which lipoperoxidation had been induced by administering radical initiator AAPH into the abdominal cavity. It should 20 be noted that the antioxidant actions referred to with the present invention are actions preventing or controlling DNA damage, cell mutation, morphological transformation, oxidative cell damage including cell death and the like emanating from irreversible oxidative reactions of cellular components due to free radicals or lipid peroxides, and broadly include free radical scavenging activity and lipoperoxidation-suppressing 25 activity.

As a result, both the Test Groups 1-2 and 1-3 showed significant suppression of increases in urinary 8-OHdG level and significant suppression of increases in the amount of lipid peroxide in the livers. Furthermore, no differences (reason for no side effects) in weight shift, feed intake, and water intake among the seven groups: Control 30 Groups 1-0 through 1-3 and Test Groups 1-1 through 1-3 were recognized in the seven days before AAPH administration.

It is understood from the above-given results that when the antioxidant-functioning waters (pharmacologic-functioning waters) according to the Test Groups 1-2 and 1-3 are consumed, oxidative damage of genetic DNA in the living

organism was controlled, and antioxidant action of suppressing lipoperoxidation was demonstrated, without influencing the contributing factors of weight shift, feed intake, and water intake, namely, the pharmacologic-functioning waters demonstrated pharmacologic function without any side effects.

5   It has been found in recent years that free radicals or lipid peroxides in living organisms are prominently responsible for oxidative stress-related disorders such as hepatic damage due to drugs or harmful substances, ischemia/reperfusion injury, circulatory system diseases such as arteriosclerosis, gastric ulcers, digestive system diseases such as gastric mucosa injury, respiratory diseases, diabetes complications (e.g.,

10   high blood pressure, cerebral infarction, heart attack, and the like), cataract, cutaneous diseases, various inflammatory diseases, neurological disorders, cancer, and aging. The oxidative stress-related disorders refer to all disorders due to free radicals or lipid peroxides in living organisms.   The lipid peroxides are generated by highly-unsaturated fatty acids, which constitutes a cell membrane or the like, becoming

15   a base and then being oxidized at the part of the unsaturated group through action of the free radicals or the like including active oxygen species.   When the cell membrane function is impaired with the lipid oxidation, cell damage develops depending on the action of the generated lipid peroxides.   The lipid peroxides generated within the living organisms may be lipid hydroperoxide (LOOH), LCHO, or free radicals generated

20   therefrom, for example, a lipid radical such as a peroxy radical $LOO\cdot$ or an alkoxy radical $LO\cdot$.

Here, involvement of free radicals or lipid peroxides including active oxygen species in the above-mentioned oxidative stress-related disorders is described.

The liver is a central organ for detoxification and metabolism and is thus easily

25   damaged by a hepatoxic factor, chemical drugs, and the like.   To establish the pathology of liver damage due to chemical intoxication or other causes, more specifically carbon tetrachloride ($CCl_4\cdot$) intoxication, for example, a cell damage mechanism due to a free radical is presented.   It is revealed that carbon tetrachloride has toxicity such as carcinogenicity or hepatic toxicity.   The principal development of toxicity has been

30   known for some time as the ($\cdot CCl_3$) radical.   This radical induces lipoperoxidation, resulting in liver damage ('Kasseisanso to Ishokudougen' written and edited by Inoue, Masayasu, Kyouritsu Shuppan Co., Ltd., p. 135-137.)   Accordingly, this radical is also responsible for such liver damage due to chemicals.   Similarly, typical symptoms due to cell damage due to active oxygen species are also seen with paraquat intoxication.

Paraquat intoxication results from generation of active oxygen in living organisms, and that active oxygen evokes lipoperoxidation, cell membrane alteration, and cell damage. In serious cases, symptoms such as digestive symptoms, liver dysfunction, and renal dysfunction irreversibly develop, ultimately leading to progressive pulmonary fibrosis, respiratory failure, and eventually death.

Ischemia/reperfusion injury (I/R) is a disorder observed when ischemia due to blood flow disruption or low perfusion generates for a definite period of time, and then resumes blood flow. It is known that active oxygen and free radicals generate in an ischemic condition and reoxygenation process, causing lipoperoxidation, cell membrane damage, and tissue damage, paradoxically expressed as exacerbation of symptoms. As mechanisms thereof, factors such as (1) post-reoxygenation oxidative stress, (2) intracellular pH variation, (3) mitochondria before and after ischemia, (4) activation of post-reoxygenation inflammation-mediated tissue, (5) intracellular Ca^{2+} level variation, (6) induction of ischemic hypoxia inducing factor, and (7) apoptosis due to post-reoxygenation caspase 3 activation are involved, and production of active oxygen in macrophages such as neutrophil and Kupffer cells is known to be prominently responsible for reperfusion injury.

, Active oxygen is involved in arterial sclerosis, influencing oxidation of low-density lipoprotein (LDL), causing lipoperoxidation of cholesterol and the like in an oxidized LDL degenerative process, and precipitating it on vascular walls. Degenerative LDL is taken in by the macrophages and made into foamy cells so that atherosclerosis is developed. Even with digestive system diseases, active oxygen species are assumed as a significant pathogenic factor of peptic ulcers. Through Helicobacter pylori or gram-negative bacillary infection, inflammatory cells infiltrate into gastrointestinal or duodenal mucosa and induce cytokine, or generate active oxygen, causing cell damage. This may be the origin of digestive system diseases such as gastric ulcers, gastric mucosal injury, and duodenal ulcers.

With diabetes, active oxygen is generated when excessive glucose produces nonenzymic glycation of proteins and then an advanced glycation endproduct (AGE). Pancreatic islet □ cells secreting insulin are attacked by active oxygen and become weak.

A cause of cataract is known to be that a crystal lens or protein is attacked and damaged by the active oxygen generated through the action of ultraviolet rays or the like.

With cutaneous diseases, active oxygen is generated in cutaneous tissue such as epidermis or cutis through irradiation of ultraviolet rays or radioactive rays, which oxidizes collagen, elastin, and the like, becoming a cause of 'pigmented spots' and 'wrinkles'. Active oxygen is also noted to be similarly responsible for skin lesions such as atopic dermatitis, cheloid, burn wounds, and skin cancer.

With cancer, DNA damage due to active oxygen produces mistranslated gene information and abnormality in regulation of gene expressions, leading to generation of cancer cells.

For aging, since defense system activity such as superoxide dismutase (SOD) decreases as age increases, active oxygen species scavenging ability decreases accordingly, thereby accelerating natural aging of organ and tissue damage. Accumulation of lipid peroxides is found in the brain and nerves of aging animals.

Accordingly, consumption of the antioxidant-functioning water according to the five groups: Test Groups 2-2 through 2-6 is proven useful as a preventive/therapeutic agent without any side effects for prevention and/or treatment of overall oxidative stress-related disorders generating from free radicals or lipid peroxides such as hepatic damage due to drugs or harmful substances, ischemia/reperfusion injury, circulatory system diseases such as arteriosclerosis, gastric ulcer, digestive system diseases such as gastric mucosal injury, respiratory diseases, diabetes complication (e.g., high blood pressure, cerebral infarction, heart attack, and the like), cataract, cutaneous disease, various inflammatory diseases, neurological disorders, cancer, aging. It should be noted that the side effects referred to with the present invention indicate a concept including useless side effects and adverse reactions in treatment for diseases. Contribution to nausea, vomiting, hair loss, fatigue, anemia, infections, impaired blood coagulation, pain of the mouth, gums, throat, and the like, diarrhea, constipation, numbness in hands and feet, harmful impact on skin and nails, cold symptoms, swelling, dependency, abuse, teratogenicity, rebound when discontinued, carcinogenesis, radical chain reactions (even if a free radical or lipid peroxide is instantaneously scavenged, it is changed into a radical itself and adversely affects elsewhere), and the like applies to the side effects referred with the present invention.

Does antioxidant-functioning water (AOW) control adjuvant arthritis in rats?

Rat adjuvant arthritis is often used as an animal model for chronic rheumatism belonging to a category of autoimmune diseases. An autoimmune disease is a disease of unknown cause defined as being triggered by breakdown of its own cells,

able to make an autoantibody against the broken down cells or components thereof, and continue to break down its own white blood cells. The number of patients suffering from such autoimmune diseases is increasing year by year, and development of new preventative/therapeutic agents exhibiting pharmacologic function without any side effects, completely differing from stereotypical therapeutic agents inevitably accompanied with side effects, is very eagerly anticipated.

Accordingly, a pharmacological test is conducted using seven groups of eight rats/group in connection with the question 'Does antioxidant-functioning water (AOW) demonstrate pharmacologic function such as control adjuvant arthritis in rats?' when consumption of antioxidant-functioning water (AOW) is assumed. An outline of a testing protocol is given forthwith. It should be noted that with this pharmacologic test, administering adjuvant of fungus origin to rats induces arthritis. While sensitive limbs at the injection site show arthritis symptoms (primary inflammation) in several hours, it is about 10 days after injection that arthritis symptoms (secondary inflammation) can be observed over the entire body. Furthermore, the arthritis symptoms peak around 20 days after injection. Therefore, the duration for this test is set to 24 days.

(A) Outline of Testing Protocol

(A-1) Test animals and breeding environment

Eight week-old female specific pathogen free (SPF) Lewis rats purchased from Charles Rivers Laboratories Japan, Inc. were bred in preparation for seven days and then provided for testing. The rats were bred four to a cage in a SPF barrier breeding room (lighting hours: 8 AM to 6 PM, air change rate: 18 times/hour) at a room temperature of $24 \pm 3°C$ and humidity of $55 \pm 15\%$ throughout the preparatory breeding period and the testing period.

Regarding feed, all groups were freely given solid feed (MF manufactured by Oriental Yeast Co., Ltd.). Regarding water supply, deionized water was filled into feedwater bottles and freely taken in during the preparatory breeding period. Once testing began, test articles were filled into respective feedwater bottles and freely taken in. It should be noted that the feedwater bottles used during the preparatory breeding period and after testing had begun were feedwater bottles improved by our company (MiZ Co., Ltd.) so as to prevent air (oxygen) from getting mixed into the fluid in the bottles.

(A-2) Main instruments, equipment and materials, and reagents used in test

(1) Plethysmometer: Model TK-105 manufactured by Muromachi Kikai Co., Ltd.

(2) Tubercle bacillus: *M. tuberculosis* H37Ra manufactured by Wako Pure Chemical Industries, Ltd., Lot No. 2116641

(3) Liquid paraffin manufactured by Wako Pure Chemical Industries, Ltd., Lot No. EWQ1149

(A-3) Outline of test articles

(2) Purified water (used for Control Group 2-1)

(2) Catalyst-free circulating electrolyzed water (electrolyzed hydrogen water, used for Test Group 2-1)

(6) Post-electrolysis catalyst-added circulating electrolyzed water (Pd colloid catalyst (approximately 300 μg/L concentration) -containing electrolyzed hydrogen water, used for Test Group 2-2)

(4) Pre-electrolysis catalyst-added circulating electrolyzed water (concentration of the Pd colloid catalyst is approximately 600 μg/L) -containing electrolyzed hydrogen water, used for Test Group 2-3)

(5) Pre-electrolysis catalyst-added circulating electrolyzed water (concentration of the Pt colloid catalyst is approximately 300 μg/L) -containing electrolyzed hydrogen water, used for Test Group 2-4)

(6) Pre-electrolysis catalyst-added circulating electrolyzed water (concentration of the Pt/Au alloy colloid catalyst is approximately 300 μg/L) -containing electrolyzed hydrogen water, used for Test Group 2-5)

(7) Pre-electrolysis catalyst-added circulating electrolyzed water (concentration of the Pd/Au alloy colloid catalyst is approximately 300 μg/L) -containing electrolyzed hydrogen water, used for Test Group 2-6)

(A-4) Testing method, and examination and inspection items

(1) Preparation of adjuvant and induction of arthritis

Once heat-killed bacteria of *M. tuberculosis* H37Ra (manufactured by Wako Pure Chemical Industries, Ltd., Lot No. 2116641) is reduced to a fine powder using a suitable amount scale agate mortar, liquid paraffin (manufactured by Wako Pure Chemical Industries, Ltd., Lot No. EWQ1149) is added little by little and then suspended, making 3 mg/ml suspension. Each rat was fastened to a fixed base under ether anesthesia, and 0.1 ml of the made adjuvant was injected in the footpad of the right hind leg to induce arthritis. It should be noted that day of induction is defined as Day 0.

(2) Administration method

Immediately after opening a 200 mL capacity light-resistant glass bottle, 3 mL of each test article (200 mL included in the bottle) assigned to each rat in each group was forcedly administered orally to each rat using a disposable gastric sonde for twenty-four days from Day 0 to Day 23. During that period, the water in the feedwater bottle was exchanged with fresh water. In other words, once old water left in the feedwater bottle was discarded, fresh remaining water after being used for forced oral administration was gently filled up to the maximum amount of the feedwater bottle so as to remove as much of the air layer therein as possible, and a water supply tap was then set so as to freely provide water.

(3) Observation of general state

The general state was observed once daily and entered into the recording sheet.

(4) Weight measurement

Weight of the rats in each group was measured with a scale. Weight measurement dates were Days 0, 3, 8, 13, 16, and 23.

(5) Arthritis score observation

The same examiner randomly grossly observed degrees of reddening, swelling, and ankylosis in the right fore leg, left fore leg, and left hind leg of the sensitized region excluding the right hind leg, and provided scores of 0-4 points according to the criteria given below, evaluating with a total maximum of 12 points. Observation dates were the same as the weight measurement dates.

0: No symptoms recognized (nil)

1: Only one small joint of a limb, a finger or the like indicated reddening and swelling (mild)

2: Two or more small joints or relatively large joints such as wrists or ankles indicated reddening and swelling (moderate)

3: One paw or entire leg showed reddening and swelling (mild severe)

4: Overall swelling of one paw or leg had reached maximum, with ankylosis of the joints (severe)

(6) Leg volume measurement

The right hind leg volume of the rats of each group was measured using a plethysmometer. Measurement dates were the same as the weight measurement dates.

(7) Statistical processing

The obtained weights, arthritis scores, and right hind leg capacities were given as average value ± standard error for every group. In order to verify the statistical significant difference among the respective groups (n=8), statistical processing was conducted using an analysis software (Stat View, Abacus Inc., USA). Upon

5 confirmation of weight and leg volume data to be homoscedastic through analysis of variance (ANOVA), multiple comparative assay which is the Fisher's PLSD method was carried out to compare the groups. Furthermore, the arthritis score data was studied to compare the groups using Mann-Whitney's U-test. In any case, a relative risk of less than 5% (p<0.05) was found to be statistically significant.

10 (B) Disclosure of test groups and control groups

(Control Group 2-1)

A group tested for eight rats in conformity with the testing procedures described in (A-2) when activated charcoal processed water (purified water) resulting from processing Fujisawa City tap water through an activated charcoal column is

15 employed as feed water is given as Control Group 2-1.

(Test Group 2-1)

A group tested for eight rats in conformity with the testing procedures described in (A-4) when catalyst-free circulating electrolyzed water is used as feeding water, which results from subjecting 1 liter of the same purified water as with Control

20 Group 2-1 to electrolysis processing (equivalent to 2-pass electrolysis processing) for one minute using a continuous flow circulating method (volume of circulatory water: 0.8 liters) under conditions of a 1.5 L/min flow and 5A constant current, is given as Test Group 2-1.

(Test Group 2-2)

25 A group tested for eight rats in conformity with the testing procedures described in (A-4) when post-electrolysis catalyst-added circulating electrolyzed water added an amount of the Pd standard solution described with Working Examples 6 through 8 sufficient to give a Pd colloid concentration of approximately 300 μg/L is used as feeding water, which results from subjecting 1 liter of the same purified water as

30 with Control Group 2-1 to electrolysis processing (equivalent to 2-pass electrolysis processing) for one minute using a continuous flow circulating method (volume of circulatory water: 0.8 liters) under the same conditions of Test Group 2-1, is given as Test Group 2-2.

(Test Group 2-3)

A group tested for eight rats in conformity with the testing procedures described in (A-4) when pre-electrolysis catalyst-added circulating electrolyzed water added an amount of the Pd standard solution described with Working Examples 6 through 8 sufficient to give a Pd colloid concentration of approximately 600 µg/L is used

5   as feeding water, which results from subjecting 1 liter of the same purified water as with Control Group 2-0 to electrolysis processing (equivalent to 2-pass electrolysis processing) for one minute using a continuous flow circulating method (volume of circulatory water: 0.8 liters) under the same conditions of Test Group 2-1, is given as Test Group 2-3.   It should be noted that the difference between Test Group 2-2 and Test

10   Group 2-3 is when and at what concentration the precious metal colloid (Pd) was added thereto.

(Test Group 2-4)

A group tested for eight rats in conformity with the testing procedures described in (A-4) when pre-electrolysis catalyst-added circulating electrolyzed water

15   added an amount of the Pt standard solution described with Working Examples 3 through 5 sufficient to give a Pt colloid concentration of approximately 300 µg/L is used as feeding water, which results from subjecting 1 liter of the same purified water as with Control Group 2-0 to electrolysis processing (equivalent to 2-pass electrolysis processing) for one minute using a continuous flow circulating method (volume of

20   circulatory water: 0.8 liters) under the same conditions of Test Group 2-1, is given as Test Group 2-4.

(Test Group 2-5)

A group tested for eight rats in conformity with the testing procedures described in (A-5) when pre-electrolysis catalyst-added circulating electrolyzed water

25   added an amount of the same Pt/Au alloy colloid-containing solution as in Working Example 90 sufficient to give a colloid concentration of approximately 300 µg/L is used as feeding water, which results from subjecting 1 liter of the same purified water as with Control Group 2-1 to electrolysis processing (equivalent to 2-pass electrolysis processing) for one minute using a continuous flow circulating method (volume of

30   circulatory water: 0.8 liters) under the same conditions of Test Group 2-1, is given as Test Group 2-5.

(Test Group 2-6)

A group tested for eight rats in conformity with the testing procedures described in (A-6) when pre-electrolysis catalyst-added circulating electrolyzed water

added an amount of the same Pd/Au alloy colloid-containing solution as in Working Example 93 sufficient to give a colloid concentration of approximately 300 μg/L is used as feeding water, which results from subjecting 1 liter of the same purified water as with Control Group 2-1 to electrolysis processing (equivalent to 2-pass electrolysis

5   processing) for one minute using a continuous flow circulating method (volume of circulatory water: 0.8 liters) under the same conditions of Test Group 2-1, is given as Test Group 2-6.

(C) Test Results

Weight

10   FIG. 48 and Table 9, which compare seven groups: Control Group 2-1 and Test Groups 2-1 through 2-6 for weight shift throughout the entire testing period, show the influence of consumption of precious metal colloid catalyst-containing electrolyzed hydrogen water (AOW) upon rat weight shift. It should be noted that in order to guarantee viewability of lines in FIG. 48, display of the standard error has been

15   omitted.

[Table 9]

★

The rat weight shifts given in FIG. 48 and Table 9 show weight improvement tendencies for six groups: Test Groups 2-1 through 2-6 throughout most of the testing in

20   comparison to Control Group 2-1. Particularly, the five groups: Test Groups 2-1 through 2-4 and 2-6 excluding Test Group 2-5 on Day 13, and the two groups: Test Groups 2-3 and 2-6 on Day 21 all showed significant weight improvement.

State observation

State observation throughout the entire testing period did not find abnormal

25   changes other than arthritis symptoms in all of the groups.

Arthritis scores

FIG. 49 and Table 10, which compare seven groups: Control Group 2-1 and Test Groups 2-1 through 2-6 for arthritis score transition throughout the entire testing period, show the influence of consumption of precious metal colloid catalyst-containing

30   electrolyzed hydrogen water (AOW) upon arthritis score transition. It should be noted that in order to guarantee viewability of lines in FIG. 49, display of the standard error has been omitted.

[Table 10]

★

In the arthritis score transitions given in FIG. 49 and Table 10, while Control Group 2-1 did not show arthritis symptoms until Day 8, severe arthritis symptoms were recognized after Day 13. On the observation dates between Days 13 and 23, the five groups: Test Groups 2-2 through 2-6 excluding Test Group 2-1 showed slight or significant suppression of rising of arthritis scores in comparison to Control Group 2-1. Particularly, the three groups: Test Groups 2-2, 2-3, and 2-5 on Day 13, the four groups: Test Groups 2-2 through 2-5 on Day 16, and the Test Group 2-3 on Day 23 all showed significant suppression of rising in arthritis scores.

Volume of sensitized limb (right hind leg)

FIG. 50 and Table 11, which compare seven groups: Control Group 2-1 and Test Groups 2-1 through 2-6 for sensitized limb (right hind leg) volume transition throughout the entire testing period, show the influence of consumption of precious metal colloid catalyst-containing electrolyzed hydrogen water (AOW) upon sensitized limb volume transition. It should be noted that in order to guarantee viewability of lines in FIG. 50, display of the standard error has been omitted.

[Table 11]

★

In the sensitized limb volume transition given in FIG. 50 and Table 11, although the six groups: Test Groups 2-1 through 2-6 showed slight or significant increases in sensitized limb volume until Day 8, this is regarded as nothing but incidental change. On Day 13, in comparison to Control Group 2-1, the three groups: Test Groups 2-1, 2-4, and 2-6 showed slight increases in sensitized limb volume transition, while the three groups: Test Groups 2-2, 2-3, and 2-5 began to show slight suppression of increases in sensitized limb volume transition. On Days 16, in comparison to Control Group 2-2, the five groups: Test Groups 2-1, and 2-3 through 2-6 excluding Test Group 2-2 showed slight or significant suppression of increases in sensitized limb volume, where Test Group 2-6 particularly showed significant suppression of increases in sensitized limb volume. On Days 21, in comparison to Control Group 2-1, the six groups: Test Groups 2-1 through 2-6 showed slight or significant suppression of increases in sensitized limb volume, where Test Groups 2-2, and 2-4 through 2-6 particularly showed significant suppression of increases in sensitized limb volume. On Days 23, in comparison to Control Group 2-1, the six groups: Test Groups 2-1 through 2-6 showed slight or significant suppression of increases in sensitized limb volume, where the four groups: Test Groups 2-3 through 2-6

particularly showed significant suppression of increases in sensitized limb volume.

(D) Examination of results

Benefits in prevention of the incidence of adjuvant arthritis from forced oral administration of the test articles and free intake were compared and studied using a
5 model animal in which adjuvant was injected in the footpad of the right hind leg to induce arthritis.

As a result, all of the five groups: Test Groups 2-2 through 2-6 showed slight or significant weight improvement, slight or significant suppression of rising in arthritis scores, and significant suppression of increases in sensitized limb volume. Respective
10 inspection items from throughout the entire testing period were sorted according to the number of significant differences (favorable to have more times of significant differences). Regarding weight shift, the two groups: Test Group 2-3 and 2-6 showed favorable weight improvement; regarding arthritis score transition, the three groups: Test Groups 2-2, 2-3, and 2-5 showed favorable suppression of increases in arthritis
15 scores; and regarding sensitized limb volume transition, the three groups: Test Groups 2-4 through 2-6 showed favorable suppression of increases in sensitized limb volume. It should be noted that state observation throughout the entire testing period did not find abnormal changes other than arthritis symptoms in all of the groups (reason for no side effects).

20 It is understood from these results that when the antioxidant-functioning waters (pharmacologic-functioning waters) according to the five groups: Test Groups 2-2 through 2-6 were consumed, without inducing abnormal changes other than arthritis symptoms, improvement effects of weight reduction deriving from rat adjuvant arthritis, outbreak delay effect and outbreak prevention effect of rat adjuvant arthritis, and
25 increase suppression effect of sensitized limb volume deriving from rat adjuvant arthritis were achieved, namely the pharmacologic-functioning waters demonstrated pharmacologic function without any side effects.

However, consumption of the antioxidant-functioning waters (pharmacologic-functioning waters) according to the five groups: Test Groups 2-2
30 through 2-6 suggested effectiveness without any side effects against adjuvant arthritis, which is an animal model for human chronic rheumatism. In other words, consumption of the antioxidant-functioning waters according to the five groups: Test Groups 2-2 through 2-6 would prove useful as antirheumatics for preventing or treating chronic rheumatism. In addition, since human chronic rheumatism is an autoimmune

disease, and consumption of the antioxidant-functioning waters according to the five groups: Test Groups 2-2 through 2-6 indicates effectiveness against adjuvant arthritis, it would also indicate effectiveness without any side effects against other autoimmune diseases. In other words, consumption of the antioxidant-functioning waters according to the five groups: Test Groups 2-2 through 2-6 would prove useful as an anti-autoimmune disease agent for preventing or treating autoimmune diseases such as systemic lupus erythematosus (SLE), Sjogren's syndrome, scleroderma, insulin-dependant diabetes by which insulin-producing cells of the pancreas are damaged, idiopathic thrombocytopenic purpura by which blood platelets are damaged, chronic thyroiditis by which the thyroid gland is damaged, graves disease, pernicious anemia, Addison's disease, atrophic gastritis, hemolytic anemia, ulcerative colitis, myasthenia gravis by which nerve cell receptors are damaged, multiple sclerosis, noninsulin-dependent diabetes mellitus, chronic nephritis, Meniere's disease, sudden deafness, emphysema, Behcet's Syndrome, viral hepatitis, muscular dystrophy, amyotrophic lateral sclerosis (ALS) due to damage of motor nerve cells, depression due to receptor damage of brain neurons, atopic dermatitis, and pollen allergen.

Disclosure of additional working examples by DH quantitative analysis method using oxidation/reduction pigment

Additional reference examples and working examples by a DH quantitative analysis method using the above-mentioned oxidation/reduction pigment are described below.

(Reference Example 35)

Using the purified water used for Control Groups 1-0, 1-1, and 2-1 in the aforementioned pharmacologic test as test water, 1 mL of one-fortieth strength Pt standard solution that has undergone the nitrogen gas replacement described above is injected to 200 mL of this test water in a test water holding compartment using a syringe. This is then sufficiently stirred and mixed, and thereafter while visually observing the color change of the test water, a 10 g/L concentration (mole concentration by volume: 26773.8 μM) of methylene blue solution is injected a little bit at a time into the test water using a syringe. The total amount of methylene blue injected until reaching the end point was 0 mL, and the effective value of dissolved hydrogen concentration DH found by substituting each value into the above-given Equation 7 was 0 (mg/L). Each physical property value and effective value of the dissolved hydrogen concentration DH of the test water according to this Reference Example 35 are shown in

Table 12, and the effective values of the dissolved hydrogen concentration DH are shown in FIG. 51.

(Reference Example 36)

When purified water containing the Pt colloid catalyst (approximately 200 µg/L concentration) used for Control Group 1-2 in the aforementioned pharmacologic test was used as test water, and the DH quantitative analysis method was conducted through methylene blue titration as with Reference Example 35, the total amount of methylene blue solution injected until reaching the end point was 0 mL, and the effective value of dissolved hydrogen concentration DH found by substituting each value into the above-given Equation 7 was 0 (mg/L). Each physical property value and effective value of the dissolved hydrogen concentration DH of the test water according to this Reference Example 36 are shown in Table 12, and the effective values of the dissolved hydrogen concentration DH are shown in FIG. 51.

(Reference Example 37)

When purified water containing the Pd colloid catalyst (approximately 200 µg/L concentration) used for Control Group 1-3 in the aforementioned pharmacologic test was used as test water, and the DH quantitative analysis method was conducted through methylene blue titration as with Reference Example 35, the total amount of methylene blue solution injected until reaching the end point was 0 mL, and the effective value of dissolved hydrogen concentration DH found by substituting each value into the above-given Equation 7 was 0 (mg/L). Each physical property value and effective value of the dissolved hydrogen concentration DH of the test water according to this Reference Example 37 are shown in Table 12, and the effective values of the dissolved hydrogen concentration DH are shown in FIG. 51.

(Reference Example 38)

When the catalyst-free circulating electrolyzed water (electrolyzed hydrogen water) used for Test Groups 1-1 and 1-2 in the aforementioned pharmacologic test was used as test water, and the DH quantitative analysis method was conducted through methylene blue titration as with Reference Example 35, the total amount of methylene blue solution injected until reaching the end point was 6.4 mL, and the effective value of dissolved hydrogen concentration DH found by substituting each value into the above-given Equation 7 was 1.71 (mg/L). Each physical property value and effective value of the dissolved hydrogen concentration DH of the test water according to this Reference Example 38 are shown in Table 12, and the effective values of the dissolved

hydrogen concentration DH are shown in FIG. 51.

(Working Example 96)

When electrolyzed hydrogen water containing pre-electrolysis catalyst-added circulating electrolyzed water (concentration of the Pd colloid catalyst is approximately 200 μg/L) used for Test Group 1-2 in the aforementioned pharmacologic test was used as test water, and the DH quantitative analysis method was conducted through methylene blue titration as with Reference Example 35, the total amount of methylene blue solution injected until reaching the end point was 6.3 mL, and the effective value of dissolved hydrogen concentration DH found by substituting each value into the above-given Equation 7 was 1.69 (mg/L). Each physical property value and effective value of the dissolved hydrogen concentration DH of the test water according to this Working Example 96 are shown in Table 12, and the effective values of the dissolved hydrogen concentration DH are shown in FIG. 51.

(Working Example 97)

When electrolyzed hydrogen water containing pre-electrolysis catalyst-added circulating electrolyzed water (concentration of the Pd colloid catalyst is approximately 200 μg/L) used for Test Group 1-3 in the aforementioned pharmacologic test was used as test water, and the DH quantitative analysis method was conducted through methylene blue titration as with Reference Example 35, the total amount of methylene blue solution injected until reaching the end point was 6.4 mL, and the effective value of dissolved hydrogen concentration DH found by substituting each value into the above-given Equation 7 was 1.71 (mg/L). Each physical property value and effective value of the dissolved hydrogen concentration DH of the test water according to this Working Example 97 are shown in Table 12, and the effective values of the dissolved hydrogen concentration DH are shown in FIG. 51.

(Working Example 98)

When electrolyzed hydrogen water containing post-electrolysis catalyst-added circulating electrolyzed water (concentration of the Pd colloid catalyst is approximately 300 μg/L) used for Test Group 2-2 in the aforementioned pharmacologic test was used as test water, and the DH quantitative analysis method was conducted through methylene blue titration as with Reference Example 35, the total amount of methylene blue solution injected until reaching the end point was 6.4 mL, and the effective value of dissolved hydrogen concentration DH found by substituting each value into the above-given Equation 7 was 1.71 (mg/L). Each physical property value and effective

value of the dissolved hydrogen concentration DH of the test water according to this Working Example 98 are shown in Table 12, and the effective values of the dissolved hydrogen concentration DH are shown in FIG. 51.

(Working Example 99)

5   When electrolyzed hydrogen water containing pre-electrolysis catalyst-added circulating electrolyzed water (concentration of the Pd colloid catalyst is approximately 600 μg/L) used for Test Group 2-3 in the aforementioned pharmacologic test was used as test water, and the DH quantitative analysis method was conducted through methylene blue titration as with Reference Example 35, the total amount of methylene 10   blue solution injected until reaching the end point was 6.7 mL, and the effective value of dissolved hydrogen concentration DH found by substituting each value into the above-given Equation 7 was 1.79 (mg/L). Each physical property value and effective value of the dissolved hydrogen concentration DH of the test water according to this Working Example 99 are shown in Table 12, and the effective values of the dissolved 15   hydrogen concentration DH are shown in FIG. 51.

(Working Example 100)

       When electrolyzed hydrogen water containing pre-electrolysis catalyst-added circulating electrolyzed water (concentration of the Pd colloid catalyst is approximately 300 μg/L) used for Test Group 2-4 in the aforementioned pharmacologic test was used as 20   test water, and the DH quantitative analysis method was conducted through methylene blue titration as with Reference Example 35, the total amount of methylene blue solution injected until reaching the end point was 6.3 mL, and the effective value of dissolved hydrogen concentration DH found by substituting each value into the above-given Equation 7 was 1.69 (mg/L). Each physical property value and effective 25   value of the dissolved hydrogen concentration DH of the test water according to this Working Example 100 are shown in Table 12, and the effective values of the dissolved hydrogen concentration DH are shown in FIG. 51.

(Working Example 101)

       When electrolyzed hydrogen water containing pre-electrolysis catalyst-added 30   circulating electrolyzed water (concentration of the Pt/Au alloy colloid catalyst is approximately 300 μg/L) used for Test Group 2-5 in the aforementioned pharmacologic test was used as test water, and the DH quantitative analysis method was conducted through methylene blue titration as with Reference Example 35, the total amount of methylene blue solution injected until reaching the end point was 6.4 mL, and the

effective value of dissolved hydrogen concentration DH found by substituting each value into the above-given Equation 7 was 1.71 (mg/L). Each physical property value and effective value of the dissolved hydrogen concentration DH of the test water according to this Working Example 101 are shown in Table 12, and the effective values of the

5   dissolved hydrogen concentration DH are shown in FIG. 51.

(Working Example 102)

    When electrolyzed hydrogen water containing pre-electrolysis catalyst-added circulating electrolyzed water (concentration of the Pd/Au alloy colloid catalyst is approximately 300 µg/L) used for Test Group 2-6 in the aforementioned pharmacologic

10   test was used as test water, and the DH quantitative analysis method was conducted through methylene blue titration as with Reference Example 35, the total amount of methylene blue solution injected until reaching the end point was 6.4 mL, and the effective value of dissolved hydrogen concentration DH found by substituting each value into the above-given Equation 7 was 1.71 (mg/L). Each physical property value and

15   effective value of the dissolved hydrogen concentration DH of the test water according to this Working Example 102 are shown in Table 12, and the effective values of the dissolved hydrogen concentration DH are shown in FIG. 51.

[Table 12]

★

20   According to Table 12, it is understood that the antioxidant-functioning water (pharmacologic-functioning water) according to the Test Groups 1-2, 1-3, and 2-2 through 2-6 is water in which an amount of hydrogen greater than the saturation concentration (when converted to the effective value of the dissolved hydrogen concentration value) is dissolved under atmospheric pressure. Incidentally, the

25   dissolved hydrogen saturation concentration under atmospheric pressure (water temperature: 20 °C) is approximately 1.6 mg/L.

    Description of the embodiments herein has been made to facilitate understanding of the present invention and is not intended to limit the invention in any way. Accordingly, each element disclosed in the above embodiments may include

30   all possible design modifications and equivalents as falls within the technical scope of the invention.

    More specifically, in the descriptions of the embodiments, reference examples, and working examples of the present invention, methylene blue was shown as an example of an oxidation/reduction pigment, however, the oxidation/reduction pigment is

not limited to this. For example, acid yellow 3, acid yellow 23, acid yellow 25, acid yellow 36, acid orange 5, acid orange 6, acid orange 7, acid orange 10, acid orange 19, acid orange 52, acid green 16, acid green 25, acid violet 43, acid brown 2, acid black 1, acid black 31, acid blue 3, acid blue 9, acid blue 40, acid blue 45, acid blue 47, acid blue

5 59, acid blue 74, acid blue 113, acid blue 158, acid red 1, acid red 2, acid red 14, acid red 18, acid red 27, acid red 37, acid red 51, acid red 52, acid red 87, acid red 88, acid red 91, acid red 92, acid red 94, acid red 95, acid red 111, solvent yellow 2, solvent black 3, solvent blue 7, solvent blue 11, solvent blue 25, solvent red 3, solvent red 19, solvent red 23, solvent red 24, solvent red 73, direct yellow 1, direct yellow 9, direct yellow 12, direct

10 yellow 59, direct green 1, direct green 6, direct green 59, direct brown 1, direct brown 6, direct black 4, direct black 22, direct black 38, direct blue 1, direct blue 6, direct blue 53, direct blue 86, direct blue 106, direct red 2, direct red 28, direct red 79, dispersed yellow 3, dispersed yellow 5, dispersed yellow 8, dispersed yellow 42, dispersed yellow 60, dispersed yellow 64, dispersed orange 3, dispersed orange 30, dispersed violet 26,

15 dispersed violet 28, dispersed blue 1, dispersed blue 26, dispersed red 1, dispersed red 4, dispersed red 60, dispersed red 65, dispersed red 73, vat yellow 2, vat green 1, vat green 3, vat brown 1, vat brown 3, vat black 25, vat blue 1, vat blue 4, vat blue 20, vat red 10, vat red 41, pigment yellow 1, pigment yellow 3, pigment yellow 10, pigment yellow 12, pigment yellow 13, pigment yellow 14, pigment yellow 17, pigment yellow 24, pigment

20 yellow 55, pigment yellow 81, pigment yellow 83, pigment yellow 93, pigment yellow 94, pigment yellow 95, pigment yellow 97, pigment yellow 98, pigment yellow 99, pigment yellow 108, pigment yellow 109, pigment yellow 110, pigment yellow 116, pigment yellow 117, pigment yellow 138, pigment yellow 151, pigment yellow 154, pigment orange 5, pigment orange 13, pigment orange 14, pigment orange 16, pigment orange 36,

25 pigment orange 38, pigment orange 40, pigment orange 43, pigment green 4, pigment green 7, pigment green 8, pigment green 10, pigment green 36, pigment violet 1, pigment violet 3, pigment violet 19, pigment violet 23, pigment violet 33, pigment brown 25, pigment black 1, pigment blue 2, pigment blue 15, pigment blue 16, pigment blue 17, pigment blue 18, pigment blue 24, pigment red 1, pigment red 3, pigment red 5, pigment

30 red 9, pigment red 22, pigment red 38, pigment red 48:1, pigment red 48:2, pigment red 48:3, pigment red 48:4, pigment red 49:1, pigment red 52:1, pigment red 53:1, pigment red 57:1, pigment red 60, pigment red 63:2, pigment red 64:1, pigment red 81, pigment red 83, pigment red 88, pigment red 112, pigment red 122, pigment red 123, pigment red 144, pigment red 146, pigment red 149, pigment red 151, pigment red 166, pigment

red 168, pigment red 170, pigment red 174, pigment red 175, pigment red 176, pigment red 177, pigment red 178, pigment red 179, pigment red 185, pigment red 187, pigment red 208, food yellow 3, food green 3, food red 6, food red 17, basic yellow 1, basic yellow 2, basic yellow 11, basic orange 1, basic orange 11, basic green 4, basic violet 3, basic violet

5   4, basic violet 10, basic violet 14, basic brown 1, basic blue 1, basic blue 3, basic blue 9, basic blue 24, basic red 1, basic red 2, basic red 5, basic red 9, basic red 18, mordant yellow 1, mordant yellow 3, mordant orange 1, mordant violet 26, mordant black 11, mordant blue 13, mordant blue 29, mordant red 3, mordant red 11, mordant red 15, reactive yellow 2, reactive yellow 3, reactive yellow 17, reactive orange 1, reactive

10   orange 2, reactive orange 16, reactive violet 2, reactive black 5, reactive blue 2, reactive blue 5, reactive blue 7, reactive blue 19, reactive red 1, reactive red 3, reactive red 6, reactive red 17, reactive red 22, and reactive red 41 may also be favorably used as a pigment with a prefix. In addition, acridine yellow G, alizarin, indamine, indoaniline, indocyanine green, urothion, urobilin, p-ethoxychrysoidne hydrochloride, m-cresol

15   purple, o-cresol phthalein, cresol red, chloroethanoic acid, chlorophyll (a, b, c, d), chlorophenol red, new methylene blue, neutral red, variamine blue B hydrochloride, methylviologen, pyocyanin, indigo carmine, safranine T, phenosafranine, Capri blue, Nile blue, diphenylamine, xylenecyanol, nitrodiphenylamine, ferroin, N-phenylanthranilic acid, 2,6-sodium dichloroindophenol, 4-sodium

20   diphenylaminesulfomic acid, N,N'-diphenylbenzidine, cinnavaline (antibiotics), toluylene blue, riboflavin (vitamin B2), acridine yellow G, p-ethoxychrysoidine hydrochloride, tetrazolium blue, and diformazan (reduced tetrazolium blue) may also be favorably used as a pigment with no prefix. Of these, in particular, methylviologen, variamine blue B hydrochloride, neutral red, pyocyanin, 2,6-sodium dichlorophenol,

25   4-sodium diphenylamine sulfonic acid, N,N'-diphenylbenzidine, cinnavalune (antibiotics), and toluylene blue may also be favorably used since their change in hue in this titration is as clear as methylene blue.

In addition, in the descriptions of the embodiments, reference examples, and working examples of the present invention, platinum (Pt) was shown as an example of a

30   precious metal catalyst which is used for dissolved hydrogen concentration quantitative analysis; however, the precious metal catalyst that can be used for dissolved hydrogen concentration quantitative analysis is not limited to this. For example, platinum, palladium, rhodium, iridium, ruthenium, gold, silver, or rhenium, along with the respective salts thereof, alloy chemical compounds, or colloidal particles themselves

such as complex chemical compounds, as well as mixtures thereof are available.

Finally, while in the description of this embodiment of the present invention, hepatic/renal damage, ischemia/reperfusion injury, circulatory system diseases such as arteriosclerosis, gastric ulcer, digestive system diseases such as gastric mucosa injury, respiratory diseases, diabetes complication (e.g., high blood pressure, cerebral infarction, heart attack, and the like), cataract, cutaneous disease, various inflammatory diseases, neurological disorders, cancer, aging, and the like have been exemplified as oxidative stress-related disorders due to free radicals or lipid peroxide, they are not limited these. In other words, they include not only oxidative stress-related disorders currently evident to be diseases involving oxidative cell damage derived from free radicals or lipid peroxide, but also diseases involving oxidative cell damage derived from free radicals or lipid peroxide to which the pharmacologic-functioning water according to the present invention evidently applicable, more specifically, all diseases specified as 'specified diseases' by the Ministry of Health, Labor and Welfare, for example.

TABLE 1

BASIC DATA FOR EACH WATER

PHYSICAL PROPERTIES FOR WATER WITHOUT HYDROGEN INCLUSION	pH	ORP [mV]	EC [mS/m]	DO [mg/L]	DH [mg/L]	T [°C]
ACTIVATED CHARCOAL PROCESSED WATER	7.31	308	16.15	8.65	0.000	22.2
ORGANO PURIFIED WATER	6.00	395	0.11	4.52	0.000	23.3
evian (REFRIGERATED)	7.30	407	56.30	9.76	0.000	12.5
PHYSICAL PROPERTIES WITH ONE-TIME ELECTROLYSIS	pH	ORP [mV]	EC [mS/m]	DO [mg/L]	DH [mg/L]	T [°C]
ACTIVATED CHARCOAL PROCESSED WATER	9.54	-735	22.30	3.22	0.900	27.5
ORGANO PURIFIED WATER (not 5A)	10.48	-760	5.60	4.45	0.425	24.2
evian (REFRIGERATED)	7.48	-530	56.10	5.25	0.460	15.7
PHYSICAL PROPERTIES WITH BUFFERED ELECTROLYSIS (30 MIN)	pH	ORP [mV]	EC [mS/m]	DO [mg/L]	DH [mg/L]	T [°C]
ACTIVATED CHARCOAL PROCESSED WATER	11.00	-850	42.80	1.76	1.332	25.8
ORGANO PURIFIED WATER (not 5A)	11.15	-850	52.30	0.94	1.374	31.9
evian (REFRIGERATED)	7.72	-635	45.10	1.46	1.157	24.2
PHYSICAL PROPERTIES WITH HYDROGEN GAS BUBBLING (30 MIN)	pH	ORP [mV]	EC [mS/m]	DO [mg/L]	DH [mg/L]	T [°C]
ACTIVATED CHARCOAL PROCESSED WATER	8.30	-585	17.97	1.67	1.070	23.6
ORGANO PURIFIED WATER	6.40	-550	0.22	1.75	1.090	23.4
evian (REFRIGERATED)	8.25	-765	50.7	2.59	0.89	21.3
ACTIVATED CHARCOAL PROCESSED WATER (by NaOH)	11.00	-836	33.50	1.55	0.910	20.9
PHYSICAL PROPERTIES WITH ELECTROLYSIS IN ELECTROLYZED WATER GENERATION APPARATUS	pH	ORP [mV]	EC [mS/m]	DO [mg/L]	DH [mg/L]	T [°C]
ALKALINE ELECTROLYZED WATER (NORMALLY EQUIPPED ACTIVATED CHARCOAL)	9.34	60	14.78	8.00	0.163	20.7

TABLE 2

SAMPLE NO.	BASE WATER 6.86				BASE WATER 9.18			
	i	ii	iii	iv	v	vi	vii	viii
pH	7.0	7.0	7.1	7.1	9.1	9.1	9.5	9.5
ORP (mV)	186	186	-625	-624	130	130	-745	-745
Pt CONCENTRATION (μg/L)	0	192	0	192	0	192	0	192
WATER TEMP (°C)	20	20	20	20	20	20	20	20

TABLE 3

	pH	ORP [mV]	EC [mS/m]	WATER TEMP [°C]	DO [mg/L]	DH MEASURED [mg/L]	DH EFFECTIVE [mg/L]
REFERENCE EXAMPLE 17	9.8	-171	17	21.6	2.67	0.18	0.03
REFERENCE EXAMPLE 18	7.2	-623	99	21.2	0.02	1.34	1.66
WORKING EXAMPLE 20	7.0	-616	99	22.4	1.00	1.06	1.58
WORKING EXAMPLE 21	9.2	-721	46	21.6	1.60	1.03	1.34
WORKING EXAMPLE 22	4.5	-446	64	21.7	1.53	0.81	1.69
WORKING EXAMPLE 23	7.1	-650	98	22.3	0.44	1.36	2.57
WORKING EXAMPLE 24	9.6	-764	54	22.3	0.45	2.20	3.29
WORKING EXAMPLE 25	4.7	-490	67	22.3	0.39	1.69	3.32

TABLE 4

	pH	ORP [mV]	EC [mS/m]	WATER TEMP [°C]	DH EFFECTIVE [mg/L]
REFERENCE EXAMPLE 17	9.8	-171	17	21.6	0.03
REFERENCE EXAMPLE 18	7.2	-623	99	21.2	1.66
WORKING EXAMPLE 78	7.1	-650	98	22.3	2.09
WORKING EXAMPLE 79	7.1	-650	98	22.3	2.28
WORKING EXAMPLE 80	7.8	-645	15	22.3	2.60
WORKING EXAMPLE 81	8.9	-707	15	18.0	2.84
WORKING EXAMPLE 82	7.4	-605	14	18.0	3.21

TABLE 5

STUDENT'S t-TEST: TEST RESULT USING TWO SAMPLES ASSUMING
EQUIVALENT VARIANCE (RELATIVE RISK : 0.1%)

	ANTIOXIDANT-FUNCTIONING WATER	PURIFIED WATER
AVERAGE LIFE SPAN	20.05051	17.74737
DISPERSION	16.66069	14.97805
NUMBER OF OBSERVED SAMPLES	99	95
POOLED DISPERSION	15.83690	
DIFFERENCE FROM HYPOTHESIS AVERAGE	0	
DEGREE OF FREEDOM	192	
t	4.02961	
P(T<=t) (ONE –SIDE)	0.00004	
BOUNDARY VALUE FOR t (ONE –SIDE)	3.13325	
P(T<=t) (TWO SIDES)	8.0467E-05	
BOUNDARY VALUES FOR t (TWO-SIDES)	3.34199	

TEST RESULT:

SINCE THE RESULT IS t = 4.03 > TWO-SIDED BOUNDARY VALUES FOR t (3.34)
BASED ON THE RELATIVE RISK OF 0.1%, THE NULL HYPOTHESIS THAT
'AVERAGE LIFE SPANS OF A GROUP THAT USES ANTIOXIDANT-FUNCTIONING
WATER AS FEED WATER AND A GROUP THAT USES PURIFIED WATER AS FEED
WATER ARE EQUAL TO EACH OTHER' IS REJECTED. ACCORDINGLY, THE
AVERAGE LIFE SPAN (20.05 DAYS) OF THE GROUP THAT USES ANTIOXIDANT-
FUNCTIONING WATER AS FEED WATER IS ONLY 2.3 DAYS LONGER THAN THE
AVERAGE LIFE SPAN (17.75 DAYS) OF THE GROUP THAT USES PURIFIED WATER
AS FEED WATER. THIS IS A SIGNIFICANT DIFFERENCE (t(192) = 4.03, SD = 0.57,
AND P < 0.001).

TABLE 6

RELATIVE INTENSITY OF DMPO-OH

TYPE OF SPECIMEN	RELATIVE INTENSITY
1 : N_2	1
2 : H_2	0.29
3 : H_2 + Pd + PVP	0.1
4 : N_2 + Pd + PVP	0.9

TABLE 7

GROUP NAME	n (NUMBER OF RATS)	8-OHdG (ng/mL) MEAN VALUE ± STANDARD ERROR	RELATIVE RISK		
			vs. CONTROL GROUP 1-1	vs. CONTROL GROUP 1-2	vs. CONTROL GROUP 1-3
CONTROL GROUP 1-0	10	13.59±0.51	—	—	—
CONTROL GROUP 1-1	10	19.84±0.59	—	—	—
CONTROL GROUP 1-2	10	19.43±0.72	n.s.	—	n.s.
CONTROL GROUP 1-3	10	19.06±0.48	n.s.	n.s.	—
TEST GROUP 1-1	10	18.08±0.40	*	n.s.	n.s.
TEST GROUP 1-2	10	16.55±0.50	***	**	**
TEST GROUP 1-3	10	16.69±0.80	**	*	*

SIGNIFICANT DIFFERENCE INDICATORS:

n.s. : not significant

* : significant ($p < 0.05$)

** : significant ($p < 0.01$)

*** : significant ($p < 0.001$)

TABLE 8

GROUP NAME	n (NUMBER OF RATS)	TBARS (nmol/mg protein) MEAN VALUE ± STANDARD ERROR	RELATIVE RISK vs. CONTROL GROUP 1-1	vs. CONTROL GROUP 1-2	vs. CONTROL GROUP 1-3
CONTROL GROUP 1-0	10	0.148±0.009	—	—	—
CONTROL GROUP 1-1	10	0.719±0.025	—	—	—
CONTROL GROUP 1-2	10	0.689±0.036	n.s.	—	n.s.
CONTROL GROUP 1-3	10	0.684±0.037	n.s.	n.s.	—
TEST GROUP 1-1	10	0.519±0.027	***	**	**
TEST GROUP 1-2	10	0.479±0.017	***	***	***
TEST GROUP 1-3	10	0.461±0.009	***	***	***

SIGNIFICANT DIFFERENCE INDICATORS:

n.s. : not significant

* : significant ($p < 0.05$)

** : significant ($p < 0.01$)

*** : significant ($p < 0.001$)

TABLE 9

SUPPRESSION EFFECT TEST FOR ADJUVANT ARTHRITIS

(GENERAL WEIGHT DATA, FISHER'S PLSD METHOD)

GROUP NAME		CHANGE IN WEIGHT OVER TIME						
		Day 0	Day 3	Day 8	Day 13	Day 16	Day 21	Day 23
CONTROL GROUP 2-1	Mean	170.9	162.5	167.1	155.5	159.5	161.1	164.7
	SE	1.55	1.50	1.87	2.06	1.99	1.85	2.09
TEST GROUP 2-1	Mean	172.3	166.9	171.2	163.2	163.8	165.9	170.2
	SE	1.53	2.11	2.44	2.08	2.18	2.30	2.48
	p	0.597	0.124	0.219	0.029*	0.212	0.147	0.118
TEST GROUP 2-2	Mean	173.5	166.9	170.5	162.5	165.0	164.9	169.6
	SE	1.72	2.39	2.88	2.82	1.91	2.43	2.45
	p	0.329	0.124	0.307	0.046*	0.112	0.248	0.159
TEST GROUP 2-3	Mean	172.3	167.1	167.7	162.9	165.8	167.9	171.5
	SE	1.26	1.38	1.50	0.84	1.67	2.02	2.49
	p	0.598	0.108	0.845	0.035*	0.074	0.040*	0.053
TEST GROUP 2-4	Mean	174.0	168.9	172.4	163.3	163.6	164.0	167.1
	SE	1.59	1.96	1.84	2.73	2.78	3.06	2.84
	p	0.245	0.028*	0.115	0.026*	0.242	0.377	0.495
TEST GROUP 2-5	Mean	170.5	164.3	166.1	158.7	164.0	163.4	167.3
	SE	1.83	2.01	2.66	2.41	2.85	2.11	2.49
	p	0.887	0.512	0.763	0.359	0.198	0.473	0.463
TEST GROUP 2-6	Mean	170.1	166.1	171.8	163.5	165.4	169.4	171.3
	SE	2.68	2.90	3.11	3.58	4.08	3.20	2.86
	p	0.783	0.202	0.157	0.023*	0.093	0.013*	0.061

SIGNIFICANT DIFFERENCE INDICATORS: *   :significant ($p < 0.05$)

                              **   :significant ($p < 0.01$)

                              ***   :significant ($p < 0.001$)

TABLE 10

SUPPRESSION EFFECT TEST FOR ADJUVANT ARTHRITIS
(GENERAL ARTHRITIS SCORE DATA, MANN-WHITNEY'S U-TEST)

GROUP NAME		CHANGE IN SCORE OVER TIME						
		Day 0	Day 3	Day 8	Day 13	Day 16	Day 21	Day 23
CONTROL GROUP 2-1	Mean	0.0	0.0	0.0	8.5	11.5	11.4	11.6
	SE	0.00	0.00	0.00	0.42	0.27	0.32	0.18
TEST GROUP 2-1	Mean	0.0	0.0	0.0	7.1	11.5	11.6	11.8
	SE	0.00	0.00	0.00	0.58	0.27	0.26	0.25
	p	-	-	-	0.1415	>.9999	0.6365	0.4948
TEST GROUP 2-2	Mean	0.0	0.0	0.0	5.8	10.1	10.4	10.5
	SE	0.00	0.00	0.00	0.25	0.35	0.60	0.50
	p	-	-	-	0.0009***	0.0117*	0.1893	0.1415
TEST GROUP 2-3	Mean	0.0	0.0	0.0	5.4	9.0	10.3	10.3
	SE	0.00	0.00	0.00	0.53	0.85	0.75	0.49
	p	-	-	-	0.0023**	0.0087**	0.3184	0.0406*
TEST GROUP 2-4	Mean	0.0	0.0	0.0	6.0	9.8	10.6	10.6
	SE	0.00	0.00	0.00	0.89	0.77	0.60	0.38
	p	-	-	-	0.0742	0.0357*	0.3720	0.0587
TEST GROUP 2-5	Mean	0.0	0.0	0.0	5.6	10.0	10.6	10.6
	SE	0.00	0.00	0.00	0.71	0.50	0.46	0.38
	p	-	-	-	0.0074**	0.0406*	0.2271	0.0587
TEST GROUP 2-6	Mean	0.0	0.0	0.0	6.8	10.6	11.0	11.3
	SE	0.00	0.00	0.00	0.62	0.50	0.33	0.31
	p	-	-	-	0.0742	0.1722	0.4309	0.4622

SIGNIFICANT DIFFERENCE INDICATORS:

* : significant ($p < 0.05$)

** : significant ($p < 0.01$)

*** : significant ($p < 0.001$)

TABLE 11

SUPPRESSION EFFECT TEST FOR ADJUVANT ARTHRITIS.
(GENERAL SENSITIZED LIMB (RIGHT HIND LEG) VOLUME DATA, FISHER'S PLSD METHOD)

GROUP NAME		CHANGE IN SENSITIZED LIMB (RIGHT HIND LEG) VOLUME OVER TIME (ml)						
		Day 0	Day 3	Day 8	Day 13	Day 16	Day 21	Day 23
CONTROL GROUP 2-1	Mean	1.34	2.76	2.93	3.74	3.87	4.04	4.13
	SE	0.01	0.06	0.07	0.09	0.12	0.13	0.11
TEST GROUP 2-1	Mean	1.35	2.90	3.05	4.11	3.85	3.93	3.82
	SE	0.01	0.05	0.07	0.07	0.14	0.16	0.09
	p	0.431	0.071	0.447	0.029*	0.902	0.614	0.068
TEST GROUP 2-2	Mean	1.36	2.91	3.03	3.66	3.90	3.57	3.89
	SE	0.00	0.04	0.20	0.10	0.11	0.12	0.12
	p	0.069	0.047*	0.542	0.628	0.885	0.024*	0.151
TEST GROUP 2-3	Mean	1.35	2.96	3.14	3.65	3.67	3.86	3.56
	SE	0.01	0.04	0.08	0.13	0.15	0.17	0.18
	p	0.511	0.009**	0.180	0.596	0.231	0.397	0.0012**
TEST GROUP 2-4	Mean	1.36	3.04	3.11	3.88	3.77	3.61	3.60
	SE	0.01	0.07	0.11	0.11	0.14	0.17	0.14
	p	0.028	0.0003***	0.249	0.392	0.539	0.039*	0.002**
TEST GROUP 2-5	Mean	1.36	2.83	3.05	3.66	3.75	3.63	3.50
	SE	0.01	0.06	0.09	0.11	0.05	0.09	0.08
	p	0.051	0.366	0.462	0.633	0.461	0.049*	0.0004***
TEST GROUP 2-6	Mean	1.34	2.91	3.01	3.83	3.45	3.61	3.69
	SE	0.01	0.05	0.09	0.14	0.16	0.18	0.09
	p	0.792	0.047*	0.623	0.570	0.015*	0.037*	0.011**

SIGNIFICANT DIFFERENCE INDICATORS:　　*　　: significant ($p < 0.05$)

　　　　　　　　　　　　　　　　　　**　　: significant ($p < 0.01$)

　　　　　　　　　　　　　　　　　　***　: significant ($p < 0.001$)

TABLE 12

	pH	ORP (mV)	EC (mS/m)	WATER TEMP (°C)	DO (mg/L)	DH EFFECTIVE (mg/L)
REFERENCE EXAMPLE 35	7.12	357	16.9	19.0	8.34	0.00
REFERENCE EXAMPLE 36	7.20	353	16.9	19.0	8.42	0.00
REFERENCE EXAMPLE 37	7.20	353	16.9	19.0	8.44	0.00
REFERENCE EXAMPLE 38	7.40	-650	17.8	21.6	3.02	1.71
WORKING EXAMPLE 96	7.40	-655	16.7	21.6	2.83	1.69
WORKING EXAMPLE 97	7.50	-651	17.1	21.6	3.13	1.71
WORKING EXAMPLE 98	7.80	-625	17.2	22.0	3.23	1.71
WORKING EXAMPLE 99	7.34	-610	17.5	22.0	2.83	1.79
WORKING EXAMPLE 100	7.30	-652	16.4	21.6	2.70	1.69
WORKING EXAMPLE 101	7.34	-654	17.0	21.7	3.03	1.71
WORKING EXAMPLE 102	7.44	-652	17.1	21.6	2.90	1.71